University of Alberta

Characterization and Evaluation of a Commercial Pork Packaging Process

by

Kenneth Alberta Fahner

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Characterization and Evaluation of a Commercial Pork Packaging Process** submitted by **Kenneth Albert Fahner** in partial fulfillment of the requirements for the degree of **Master of Science in Food Science and Technology**.

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DEDICATION

To Mom, Dad, Kevin and Sandy

Abstract

Improvement of the quality and storage life of fresh vacuum packaged pork is essential for ensuring the competitiveness of Alberta pork in export markets. The temperature experienced by vacuum packaged pork during shipment to Japan remained below 0°C for the entire 14-day shipping time. To assess the effect of a CO_2 chill tunnel on pork quality, a controlled storage experiment with pork loins and shoulder butts was done. Samples were stored at -1 and 2°C for up to 8 and 6 weeks, respectively. The CO_2 chill tunnel had no effect on the bacteriology, vacuum storage life, retail case life, flavour attributes, or purge accumulation of vacuum packaged pork loins and butts stored under controlled conditions. Under commercial conditions the CO_2 chill tunnel lowered the temperature of pork loins by 5°C which increased the storage efficiency by 40%. Chilling the surface of packaged pork prior to boxing is essential for maintaining quality.

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1. Literature Review

1.1. INTRODUCTION

Export markets have proven to be extremely lucrative for Alberta pork producers, generating revenues over $940 million in 1995 (Canadian Meat Council, 1995). Currently, the United States and Japan represent the largest export markets for Alberta pork products. In 1996 sales of pork to Japan increased 30% to 55,626 tons (Canadian Meat Council, 1996). Until recently, Alberta pork producers have been exporting a significant amount of frozen product to Japan. However, consumers generally consider the frozen product to be of a poorer quality compared with fresh, unfrozen pork. Greater consumer acceptance and higher economic returns for fresh, unfrozen product has motivated Alberta pork producers make a concerted effort to increase the amount of fresh, unfrozen product exported to distant markets, such as Japan. For Alberta pork producers to emerge as one of the dominant exporters of fresh pork to distant markets as global competition increases, they must consistently produce a high quality product with a guaranteed storage life. Improvement of fresh pork quality and increased storage life were cited in 1991 as the key factors in elevating the competitiveness of Alberta pork in export markets (Mehr, 1991).

Modified atmosphere packaging (MAP) and vacuum packaging (VP; a form of MAP) are defined as "the enclosure of food products in high gas-barrier materials, in which the gaseous environment has been changed to slow respiration rates, reduce microbiological growth, and retard enzymatic spoilage with the intent of extending shelf life" (Young et al., 1988). Modified atmosphere packaging refers to a packaging process where gas flushing with CO_2 alone or in combination with O_2 and/or N_2 provides the change in gaseous environment. Vacuum packaging refers to a form of MAP where a vacuum around the product provides the change in gaseous environment. Modified

atmosphere packaging and VP are often referred to as 'preservative' packaging systems as they have the potential to dramatically extend the storage life of fresh, unfrozen meats by providing an anaerobic environment within the package. This extension of storage life has enabled Alberta pork producers to capitalize on profitable, export markets, such as Japan, where storage and distribution time can exceed 14 days. Without MAP, and in particular VP, export to distant markets would not be possible, because the fresh, unfrozen pork packaged aerobically would be spoiled prior to reaching the Japanese market. Vacuum packaging combined with low temperature storage has the potential to extend storage life of fresh pork for weeks past that which is possible with traditional, aerobic packaging (Smith et al., 1974; Seideman et al., 1980; Lee et al., 1985; Shay and Egan, 1987).

There have been a number of studies undertaken to determine the effectiveness of low temperature storage and VP in extending the storage life of fresh pork. However, limited work has been carried out to evaluate the effectiveness of current VP systems utilized by the Alberta pork industry. The Alberta pork industry has adopted a system developed by the Cryovac Division of W.R. Grace & Co. This technologically advanced system places the fresh, unfrozen product in a high oxygen barrier package, seals the product in vacuum, and exposes the vacuum packaged product to heat, which shrinks the package around the product. The VP product is then conveyed through a carbon dioxide chill tunnel. The VP product exits the chill tunnel with a surface temperature of approximately -1.5°C. Product is immediately boxed for shipping.

When chilled, fresh meat is stored under aerobic conditions, spoilage occurs as a result of the growth of a putrefactive aerobic microflora comprised mainly of *Pseudomonas* sp. (Ingram, 1962; Gill and Newton, 1977; Dainty and Mackey, 1992). Alternatively, when fresh meat is stored anaerobically, the microflora mainly consists of gram-positive lactic acid bacteria (LAB) (Egan et al., 1986). It is this selection for LAB

coupled with the suppression of the growth of gram-negative putrefactive organisms and low temperature storage that is primarily responsible for the extension of storage life observed in MAP and VP fresh meats.

The rate of microbial metabolism and growth are highly dependent on temperature; thus, the storage life of VP meat is also temperature dependent (Zamora and Zaritzky, 1985). The optimum storage temperature of boxed product is -1.5°C (Gill et al., 1988). The Cryovac packaging system successfully prepares Alberta pork for shipment to distant markets by selecting for the growth of LAB and reducing the surface temperature of VP product to the optimum of -1.5°C prior to boxing.

Although the Alberta pork industry is currently utilizing the Cryovac packaging system, there is little information available in the scientific literature describing its effects on the quality of fresh pork or the operating conditions required to ensure that an optimal product is produced. Of primary concern is the accumulation of purge in the vacuum package. The extent to which the product is 'crust frozen' may affect the amount of liquid or purge that accumulates in the package during storage and shipment. It is commonly thought that freezing increases the amount of purge (Penny, 1974; Jalong'O et al., 1987; Greer and Murray, 1991). However, it is not clear whether meat that is 'crust frozen' experiences enough tissue damage to cause a significant increase in purge accumulation. Japanese consumers associate large amounts of purge with low quality pork. This may reduce the competitiveness of Alberta pork in the Japanese export market, with the potential to cause severe economic losses. Japanese importers are establishing stringent guidelines with respect to safety and storage life for imported fresh pork, including limits on total microbial load of product entering the country. This will increase the challenges facing the Canadian pork industry.

This study was undertaken in an effort to evaluate the current packaging process utilized by an Alberta pork packing plant. Initially, the time/temperature parameters of

the packaging system were characterized, followed by a study of the effects of the current packaging process on the microbiology, storage life, retail case life, and sensory properties of fresh pork loins and butts. Purge accumulation in the vacuum package was also evaluated.

1.2. MEAT MICROBIOLOGY DURING CHILLED ANAEROBIC STORAGE

Muscle tissue of healthy animals is essentially sterile (Gill, 1979). Therefore, microbial contamination on the carcasses of slaughtered animals and, in turn, fabricated primal cuts is acquired during slaughter and processing. Bacteria derived from the hide, skin, soil, intestinal contents, hands of workers, and equipment are present on red meat carcasses at a level between 10^2 and 10^4 bacteria/cm^2 (Dainty and Mackey, 1992). European regulations require carcasses to be chilled to a deep tissue temperature of 7°C prior to cutting or transport in an effort to restrict microbial proliferation prior to fabrication (Gill et al., 1991b). Therefore, the final microbial condition of fresh meat reflects the overall hygiene and temperature control of the dressing, cooling, and fabrication processes (Egan and Roberts, 1987).

The microflora of freshly cut meat is comprised of a large heterogeneous population of mesophiles and psychrotrophs. In developed countries, fresh meat is held under refrigeration; thus, microbial growth is restricted to psychrotrophs that are able to grow at temperatures close to 0°C (Eddy, 1960). Fresh, chilled meat stored aerobically spoils due to the growth of gram-negative putrefactive organisms, consisting mainly of *Pseudomonas* spp. (Ingram, 1962; Gill and Newton, 1977; Dainty and Mackey, 1992). Only a fraction of the initial contaminants make up the psychrotrophic spoilage flora of anaerobically packaged, fresh chilled meat. These bacteria are *Brochothrix thermosphacta*, *Shewanella* (formerly *Alteromonas*) *putrefaciens*, organisms from the

family *Enterobacteriaceae* (Gill and Greer, 1993). The prevalence of LAB results in a greatly extended storage life for anaerobically stored meats.

Lactic acid bacteria are a group of aerotolerant, gram-positive, nonsporeforming, strictly fermentative organisms that produce lactic acid as the major end product of carbohydrate metabolism (Kandler, 1983). LAB are comprised of heterogeneous genera that include: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus,* and *Vagococcus*. *Aerococcus* and *Vagococcus* spp. are not associated with foods (Holzapfel, 1992). *Carnobacterium, Lactobacillus,* and *Leuconostoc* spp. represent the LAB that usually dominate the microflora of anaerobically stored chilled meats (Hitchener et al., 1982; Shaw and Harding, 1984; Schillinger and Lücke, 1987a,b; Borch and Molin, 1988; McMullen and Stiles, 1993). Lactic acid bacteria are divided into two large groups based on the end-products of glucose metabolism under nonlimiting conditions. Lactic acid bacteria that produce lactic acid as the sole end-product of metabolism via the glycolytic pathway are homofermentative organisms. Lactic acid bacteria that produce lactic acid, acetic acid, and/or ethanol and CO_2 via the 6-phosphogluconate pathway are termed heterofermentative. Ethanol production is favored under anaerobic conditions (Blickstad and Molin, 1984). *Leuconostoc*, *Carnobacterium*, and some *Lactobacillus* spp. are heterofermentative; all other LAB are homofermentative.

The prevalence of LAB has a preservative effect on chill stored meats. Preservation by LAB is partly due to the production of antimicrobial compounds such as lactate, hydrogen peroxide, and bacteriocins (Stiles and Hastings, 1991). In addition, LAB exhibit slower growth rates than competing spoilage organisms in anaerobically packaged, chilled meats. However, growth of LAB on fresh, chilled meats stored anaerobically results in a slow fermentation process that eventually results in spoilage due to souring (Sutherland et al., 1976; Dainty et al., 1983). Souring does not become evident

until after the maximum number of LAB has been reached; thus, chilled fresh meat packaged anaerobically has an extended storage life. However, under anaerobic conditions some LAB can be potent spoilage organisms. Some lactobacilli can produce sulphur compounds in anaerobically packaged meat (Hitchener et al., 1982; Egan et al., 1989), which results in rapid spoilage (see section 1.4).

A study carried out by Sutherland et al. (1975b) reported that 24 LAB were present in a group of 105 organisms isolated from VP beef; eleven of the LAB were classified as lactobacilli while the others were coccus-shaped lactic acid bacteria. A study by Enfors et al. (1979) found that *Lactobacillus plantarum* represented 85% of the LAB isolated from pork loins stored anaerobically for 21 days at 4°C; the remaining LAB were heterofermentative lactobacilli. The proportion of heterofermentative LAB increased to 55% of the organisms isolated after storage for 35 days (Enfors et al., 1979). This apparent shift in microbial population was confirmed by Vanderzant et al. (1982). These researchers found that the microbial flora of VP beef shifted from a mixed homo- and heterofermentative population to a predominantly heterofermentative population. Heterofermentative *Leuconostoc* spp. can also prevail in MAP fresh meat (Savell et al., 1981; Schillinger and Lücke, 1987a; Nortjé and Shaw, 1989; Jackson et al., 1992c). In contrast, other researchers have reported that homofermentative LAB can dominate the LAB population of anaerobically packaged meats (Blickstad and Molin, 1983; Schillinger and Lücke, 1987a; Borch and Molin, 1988).

Shaw and Harding (1984) found that the lactobacilli could be divided into aciduric and nonaciduric strains based on growth on acetate media (pH 5.6). *Lactobacillus sake* and *Lactobacillus bavaricus* grew on this medium and as a result they were termed aciduric. *Lactobacillus carnis* (Shaw and Harding, 1984) and *Lactobacillus divergens* (Holzapfel and Gerber, 1983) are unable to grow on this medium and they were termed

nonaciduric. In 1987, Collins et al. reclassified the nonaciduric stains as *Carnobacterium divergens* and *Carnobacterium piscicola,* respectively.

Clearly, the LAB population of anaerobically stored meats varies. The population that emerges as the dominant group of microorganisms can play a significant role in fresh meat spoilage. Certain LAB are capable of causing early or rapid spoilage of anaerobically stored meats prior to reaching maximum population. However, LAB usually serve as effective preservative agents for fresh, chilled anaerobically stored meat.

Brochothrix thermosphacta is a gram-positive, facultative anaerobe that can grow on fresh, chilled meats stored in air and in vacuum packages. However, it plays a more significant spoilage role in VP meats, because this organism does not compete well with *Pseudomonas* spp. in an aerobic environment. If it grows under aerobic conditions *B. thermosphacta* metabolizes glucose to produce acetic acid and acetoin. Acetoin is subsequently oxidized to diacetyl, which has been associated with the characteristic "dairy-like" off-odours caused by *B. thermosphacta* spoilage. Metabolism of leucine and valine by *B. thermosphacta* produces isovaleric acid and isobutyric acid, respectively. These compounds are associated with the high spoilage potential of *B. thermosphacta* (Dainty and Hibbard, 1980). Under anaerobic conditions *B. thermosphacta* produces mainly lactic acid (Davidson et al., 1968; Gardner, 1980); thus, this organism should have a low spoilage potential under anaerobic conditions. Spoilage of VP chilled meats by *B. thermosphacta* is probably due to the presence of residual oxygen in the vacuum package, resulting in the production of malodorous compounds, as with aerobic metabolism (Gill, 1986). *B. thermosphacta* is able to reach high numbers in chilled, anaerobically packaged meats, and has been reported at numbers as high as 10^6 CFU/cm^2 (Sutherland, 1975b; Dainty et al., 1979; Erichson and Molin, 1981). Under anaerobic conditions *B. thermosphacta* is unable to grow below a pH of 5.8 (Gill and Greer, 1993). However, if conditions are made less restrictive by VP high pH meat in packages with some level of

oxygen permeability, *B. thermosphacta* can grow and cause spoilage. There is a relatively high incidence of high pH (>5.8) pork compared with other red meats (Egan et al., 1986). Thus, the growth of *B. thermosphacta* could be a significant factor in the spoilage of VP pork.

Organisms from the family *Enterobacteriaceae* can grow on chilled VP and MAP meats. This large heterogeneous family of gram-negative facultative anaerobes consists of spoilage organisms as well as potentially pathogenic bacteria. *Yersinia enterocolitica* is a psychrotrophic pathogen capable of growth at 0°C and is therefore a concern in refrigerated meats. Under aerobic conditions *Enterobacteriaceae* usually do not play a role in meat spoilage, because their aerobic growth rates are slower than that of the *Pseudomonas* spp. (Gill and Greer, 1993). However, under anaerobic conditions psychrotrophic *Enterobacteriaceae* can be responsible for early spoilage of chilled meats. Psychrotrophic spoilage *Enterobacteriaceae* have been reported at levels of 10^6 CFU /cm^2 on anaerobically stored meat (Dainty et al., 1979), and these organisms have been isolated from VP and MAP meats stored below 5°C (Hanna et al., 1976; Patterson and Gibbs, 1977; Vanderzant et al., 1982; Manu-Tawiah et al., 1993). These organisms include *Enterobacter, Hafnia and Serratia* spp. On high pH meat stored at chill temperatures under vacuum *Enterobacteriaceae* can represent a significant portion of the spoilage microflora (Patterson and Gibbs, 1977; Erichsen and Molin, 1981). However, the growth of *Enterobacteriaceae* is suppressed on high pH meat stored in 100% CO_2 (Erichsen and Molin, 1981; Rousset and Renerre, 1991).

High pH meat stored anaerobically is particularly susceptible to early spoilage. The spoilage of high pH meat (pH>6.0) is obnoxious due to growth of *Shewanella putrefaciens* and psychrotrophic *Enterobacteriaceae* (Dainty and Mackey, 1992). High pH meat stored in vacuum packages at temperatures near 5°C is at risk of early spoilage. On VP high pH meats, gram-negative bacteria, including *Enterobacteriaceae*, reach much

higher numbers than in normal pH meat (5.5-5.8) and, in turn, comprise a greater proportion of the spoilage flora, resulting in off-odours and early spoilage (Egan et al., 1986). If early spoilage of fresh meats is to be avoided, proliferation of *Enterobacteriaceae* and *Shewanella putrefaciens* in VP chilled meat must be controlled by reducing storage temperature, packaging normal pH meat (pH 5.5-5.8), and limiting oxygen permeability.

Clostridium spp. are not commonly isolated from anaerobically packaged meats. However, spoilage of normal pH, anaerobically packaged meat characterized by bloated packages and off-odours has been linked to the growth of a psychrotrophic *Clostridium* spp. (Dainty et al., 1989; Kalchayanand et al., 1989). This type of spoilage is unusual because clostridia generally do not grow well in a competitive environment (Hauschild, 1989). Dainty et al. (1989) isolated clostridia from VP beef, where lactic acid bacteria were dominant. In addition, Kalchayanand et al. (1989) reported that in an environment where LAB were present in high numbers spoilage of VP beef was caused by an organism that has been tentatively classified as *Clostridium laramie* (Kalchayanand et al., 1993).

1.3. FACTORS AFFECTING THE MICROBIOLOGY AND QUALITY OF VACUUM PACKAGED MEAT

Vacuum packaging of fresh, chilled meats has revolutionized the meat industry and has allowed Alberta pork processors to access lucrative, overseas markets. Although VP fresh meat is often bathed in a liquid, or purge, which is undesirable for import customers and consumers, the benefits of VP outweigh this noted disadvantage. The benefits include: reduced evaporative losses; preserved meat colour due to the prevention of metmyoglobin formation; aging of meat in the package; and extended shelf life (Seideman and Durland, 1983).

Spoilage of fresh meat is inevitable, whether stored in a vacuum, or in air. Vacuum packaging has the potential to extend the storage life of fresh meat. The extension of storage life depends on a number of interrelated intrinsic and extrinsic factors. Intrinsic factors are the properties of the meat itself, such as glycogen concentration and pH; extrinsic factors include oxygen permeability of the package and storage temperature. Another factor that affects the acceptability of VP fresh meats is the extent of purge accumulation in the vacuum package. Controlling these factors results in the maximum storage life for VP fresh meats.

1.3.1. Intrinsic factors

Meat provides an excellent medium for bacterial growth because it has a high water activity (a_w=0.99), favorable pH (5.6-5.8), nutrients, minerals, and growth factors (Hammes et al., 1990). Consequently, meat is highly susceptible to bacterial spoilage as a result of bacterial proliferation, leading to changes in colour, odour or flavour. Aside from water, protein and fat are the major components of meat. These components are insoluble and must be degraded prior to being utilized by bacteria, so they are not available for bacterial metabolism prior to the onset of spoilage (Dainty et al., 1975; Gill and Newton, 1980). Proteolytic enzymes are not produced until the late logarithmic phase of bacterial growth (Gill and Penny, 1977), so proteolysis is considered to be a post-spoilage phenomenon (Dainty, 1982; Greer, 1989). Alternatively, bacteria utilize soluble low molecular weight compounds listed in Table 1.1 to grow on meat (Dainty et al., 1975; Gill and Newton, 1978).

Different spoilage bacteria utilize different substrates for anaerobic growth. *B. thermosphacta* utilizes glucose as an energy source, whereas *Enterobacteriaceae*

Table 1.1. Concentration of the soluble, low molecular weight components of post rigor beef.[1]

Substance	Concentration (mg/g)
Creatine	6.5
Inosine monophosphate	3.0
Glycogen	1.0
Glucose	0.1
Glucose-6-phosphate	0.2
Lactic acid	9.0
Amino acids	3.5
Dipeptides	3.0

[1]Adapted from Gill (1985)

predominately use glucose and glucose-6-phosphate, and will degrade amino acids only after carbohydrates have been exhausted (Gill and Newton, 1977). Lactic acid bacteria utilize glucose and possibly arginine (Gill and Greer, 1993), and reports of the production of H_2S under anaerobic conditions suggests that other amino acids are metabolized. Some *Lactobacillus* and *Carnobacterium* spp. can produce H_2S (Schillinger and Lücke, 1987b; Egan et al., 1989; Leisner, 1992).

The ultimate storage life of VP, chilled meat is highly dependent on pH post rigor meat for two reasons. High pH meat, which is described as dark, firm, and dry (DFD) and is caused by prolonged stress of the animal prior to slaughter, allows the rapid growth of gram-negative microorganisms with a high spoilage potential, mainly *Enterobacteriaceae* and *S. putrefaciens*. *S. putrefaciens* can cause early spoilage in anaerobically packaged meat if conditions allow for its growth (pH>6.0). Even when this organism is not prevalent in the microbial population, large amounts of H_2S are produced due to the metabolism of cysteine (Nicol et al., 1970; Gill and Newton, 1979). *B. thermosphacta* can also contribute to early spoilage on DFD meat. In addition, DFD meat has a low glucose concentration, which causes earlier metabolism of amino acids and spoilage before the maximum number of organisms is reached. High pH meat packaged under vacuum spoils rapidly (3 to 6 weeks) due to the production of putrid odours and greening of the exudate (Bem et al., 1976; Taylor and Shaw, 1977). Greening of meat exudate stored anaerobically is due to the formation of sulfmyoglobin by H_2S binding with myoglobin (Nicol et al., 1970).

Under normal pH conditions (5.4 to 5.8) VP, chilled meat eventually spoils due to metabolic end-products formed by the proliferation of LAB. Lactic acid bacteria dominate the bacterial population of DFD meat (Dainty et al., 1979; Patterson and Gibbs, 1977; Erichsen and Molin, 1981); however, *Enterobacteriaceae* can achieve numbers that are high enough to cause early spoilage.

Clearly, intrinsic factors are instrumental in determining the storage life of fresh, chilled VP meats. Conditions of high pH (pH > 5.8) and carbohydrate limitation can lead to early spoilage of VP meats resulting in economic losses, dissatisfied wholesalers, and unhappy consumers. However, fresh chilled meats stored under vacuum, exhibiting normal intrinsic characteristics (i.e., pH 5.5 to 5.8) has a greatly extended storage life, allowing the meat industry to reach economically lucrative distant markets.

1.3.2. Storage atmosphere

The extended storage life observed in VP fresh, chilled meats has been attributed to the inhibition of putrefactive aerobic microflora, resulting in a bacterial population predominately comprised of LAB. Generally, LAB do not cause spoilage until after maximum population is reached, and spoilage is characterized as relatively non-offensive. When a vacuum is pulled around a product there is a residual level of oxygen. Vacuum packaging that results in residual levels of oxygen of less than 1% is considered a good vacuum; however, oxygen at this level allows *Pseudomonas* spp. to reach high numbers (Dainty and Mackey, 1992). Dainty (1983) showed that *Pseudomonas* spp. reached levels of 10^3 to 10^6 CFU/cm^2 in VP meats. In commercial VP, packages that provide a high oxygen barrier exhibit some level of oxygen permeability. Oxygen permeability of < 5 cc/m^2/day are considered close to optimum; however, packaging films with oxygen transmission rates of up to 70 cc/m^2/day are often used, because the price of high oxygen barrier packages is inversely proportional to oxygen permeability. Growth of *Pseudomonas* spp. on commercially VP meat is most likely proportional to the oxygen permeability of the packaging film (Gill, 1985).

Aerobic spoilage organisms such as the *Pseudomonas* spp. do not grow under strict vacuum because of the lack of oxygen. The presence of bacteriostatic CO_2 derived from residual meat respiration and from the metabolism of heterofermentative LAB

inhibits the growth of putrefactive gram-negative bacteria, while the LAB are tolerant of CO_2 (Sutherland et al., 1977; Enfors et al., 1979). This results the selection for LAB which have an increased lag phase and reduced growth rate due to the bacteriostatic effects of CO_2 (Sutherland et al., 1977; Blickstad and Molin, 1984). *Pseudomonas* spp. and *S. putrefaciens* are inhibited by 10 to 20% CO_2 (Gill and Tan, 1980), *B. thermosphacta* tolerates up to 50% CO_2 (Gardner, 1980), and lactobacilli can grow in an atmosphere of 100% CO_2 (Blickstad et al., 1981). In the presence of low levels of CO_2 the growth of *B. thermosphacta, Lactobacillus* spp. and *Enterobacteriaceae* is not significantly affected (Newton et al., 1977). *B. thermosphacta* may contribute to the spoilage of VP meats by utilizing residual oxygen to produce some offensive end-products of aerobic metabolism (Gill, 1985).

Residual oxygen in the vacuum package also has an effect on the colour stability of fresh meat. VP red meats with excess levels of residual oxygen may visually spoil due to the oxidation of myoglobin to form the brownish metmyoglobin (Renerre, 1990). Oxygen concentrations below 0.1% do not oxidize myoglobin to metmyoglobin (Gill and Molin, 1991; Penney and Bell, 1993). VP meats are purplish in colour due to the deoxygenated state of myoglobin. When exposed to air the deoxymyoglobin becomes oxygenated to form the bright red oxymyoglobin. This process is referred to as "blooming". It is this "bloomed" meat that consumers are accustomed to buy in the retail store. Currently, VP technology is not heavily used at the retail level for fresh meats because consumers perceive that the purple deoxymyoglobin is an indication of meat spoilage. Consumers consider red meat colour to be the most important quality attribute (Renerre, 1990).

Storage atmosphere is one of the most important factors dictating the storage life of fresh, chilled meats. Modifying the storage atmosphere surrounding fresh meats to include reduced levels of oxygen by VP or MAP greatly enhances the storage potential of

fresh, chilled meats. Anaerobic environments inhibit the growth of putrefactive bacteria resulting in the growth of 'preservative' lactic acid bacteria that usually do not cause spoilage until after maximum population has been reached.

1.3.3. Storage temperature

As stated earlier, fabrication processes inevitably produce fresh meat that is contaminated by a heterogeneous population of bacteria. A proportion of these bacteria will be psychrotrophic spoilage organisms capable of spoiling fresh meat due to the accumulation of offensive metabolic end-products (Gill, 1986). Because the rate of bacterial metabolism is temperature dependent, the storage life of fresh, chilled meats is also temperature dependent (Zamora and Zartzky, 1985). Maximum storage life of fresh, unfrozen meat is achieved when fresh meat is stored at the lowest temperature possible without freezing the muscle tissue (Gill and Phillips, 1993). Meat begins to freeze at approximately -1°C (Grau, 1985; Lowry and Gill, 1985), but packaged meat held in boxes will not freeze if the air temperature is held above -1.5°C (Gill et al., 1988). Therefore, the optimum temperature for packaged, boxed meat is -1.5°C. At this temperature bacterial growth is slowed; however, psychrotrophic organisms will grow and cause spoilage.

As storage temperature increases, the inhibition of microbial growth by CO_2 decreases (Gill and Tan, 1980). This is probably due to the lower solubility of CO_2 at high temperatures (Gill, 1988). The inhibitory effect of lactic acid is also decreased at higher temperatures (Grau, 1981).

Storage temperature affects the composition of microflora that develops during storage of chilled, VP meats. Higher storage temperatures provide a more suitable environment for the growth of potent spoilage organisms, such as *Enterobacteriaceae* (McMullen and Stiles, 1993), resulting in a shorter storage life. Storage temperatures

above 5°C, which is not uncommon in household refrigerators and retail display cases, increase the growth of *Enterobacteriaceae* (Beebe et al., 1976). VP pork stored at 5°C had more growth of gram-negative bacteria and a shorter storage life compared with pork stored at 0°C (Egan and Shay, 1984; Egan et al., 1986).

Temperature is the single most important factor for controlling the growth of spoilage bacteria on fresh meats. As temperature increases microbial metabolism increases resulting in increased growth and increased rate of spoilage. Consequently, one of the primary goals of the meat industry is to combine low temperature storage with VP in an effort to achieve the maximum attainable storage life for fresh meats.

1.3.4. Purge accumulation in the vacuum package

VP fresh meats are often bathed in a reddish liquid, known as purge, drip, or exudate. Purge is unattractive, and is often associated with low quality fresh meats. Vacuum packaging of fresh meats greatly enhances storage life; however, VP provides an environment that is susceptible to purge accumulation. Purge accumulation occurs because there are no evaporative losses, package leakage, and the pressure of the vacuum around the product. It is commonly thought that freezing (Penny, 1974; Añón and Calvelo, 1980; Jalong'O et al., 1987; Greer and Murray, 1991), slow chilling of carcasses (Taylor, 1972), and pale, soft, exudative (PSE) meat, which is caused by rapid post-mortem muscle tissue glycolysis causing reduced water holding capacity (Taylor, 1972), account for increased purge. However, few studies have attempted to determine other factors that may be responsible for increased purge in VP meats, such as crust or surface freezing. Due to the unattractiveness of purge, and the association of excess purge with low quality meat by consumers, many researchers have included purge assessments as part of their VP and MAP storage studies. Consequently, there has been a gradual

accumulation of information pertaining to the parameters that increase purge levels in MAP and VP meats.

A number of researchers have reported increases in purge as storage time increases (Simard et al., 1985; Weakley et al., 1986; Goddard et al., 1996; Miller et al., 1996). However, Jeremiah et al. (1995) found that purge accumulation increased only during the first three weeks of a 12 week storage study and Jeremiah and Jones (1989) reported no significant increases in purge with increased storage time. Findings of the latter study were supported by Bentley et al. (1989) who found that purge did not increase in VP ground beef patties as storage time increased. Recently, purge was shown to increase in air and VP, irradiated pork chops during the first two weeks of a four week storage period, while unirradiated samples showed no significant changes in purge during storage (Zhao et al., 1996). Miller et al. (1996) reported higher purge losses for pork loins than butts, and hypothesized that the increase was due to a greater surface area to weight ratio for loins than for butts. High storage temperatures and type of packaging have been linked to greater purge accumulation in VP and MAP meats (Simard et al., 1985; Bentley et al., 1989). In most instances, VP product has greater purge accumulation compared with MAP counterparts (Simard et al., 1985; Bentley et al., 1989; Miller et al., 1996; Zhao et al., 1996). However, conflicting results have been reported. Sørheim et al. (1996) found that pork loins packaged in CO_2 have greater purge accumulations than VP pork loins. They attributed the increased purge in MAP pork to the negative impact of CO_2 on water holding capacity. Jeremiah et al. (1995) reported that purge losses were greater for pork of normal muscle quality (pH 5.5 to 5.8) than PSE pork. These results are unexpected, because PSE pork is associated with rapid drop in muscle pH after slaughter (Shand et al., 1995), resulting in decreased water holding capacity and increased drip or purge (Taylor, 1972).

Freezing of anaerobically packaged meats has been linked to increased purge. However, the rate of freezing may affect the amount of purge. Añón and Calvelo (1980) suggested that rapid freezing may cause less purge because of the formation of intracellular ice crystals. More cellular damage occurs during slow freezing due to the formation of extracellular ice crystals. Añón and Calvelo (1980) have shown that the amount of exudate or purge depends on the time required to chill the product from -1°C to -7°C (characteristic freezing time). Freezing times up to 10 minutes cause intracellular ice crystal formation, resulting in less drip loss. No studies have been reported which assess commercial rapid chilling systems that cause a crust freeze on fresh meat in less than 10 minutes. However, crust freezing (greater than 10 minutes) of hot boned VP pork loins by submersion at -20°C in propylene glycol for 1 hour increased purge, while crust freezing in a blast freezer at -30°C for 1 hour or normal chilling at 2°C had no effect on amount of purge (Weakley et al., 1986). Similar results were reported for hot boned pork loins and shoulders chilled in a CO_2 chill tunnel (-79°C) that resulted in a crust freeze. Product subjected to a CO_2 chill tunnel has greater purge accumulation compared with normally chilled (2°C) non-hot-boned product (Wiley et al., 1989). Currently, the two reports cited above represent the only research published that observed the effects of crust freezing on purge accumulation in VP fresh meat.

Japanese importers of Alberta pork are very concerned about excessive purge; however, purge accumulation in VP meats is unavoidable. For meat that is DFD there is a lower amount of purge than for meat of normal or PSE quality; however, the deleterious microbial consequences linked to DFD meat probably outweigh the benefits of lower purge. It appears that PSE meat is the main culprit contributing to high levels of purge in VP meats. Research should be continued to determine various means to reduce the incidence of PSE meats. Muscle tissue of stress susceptible pigs often experiences rapid *post-mortem* conversion of muscle glycogen to lactic acid which results in low *post-*

mortem muscle pH, decreased water holding capacity and increased purge levels for primal, sub primal, and retail meat cuts.

1.4. SPOILAGE OF CHILLED VACUUM PACKAGED MEAT BY LAB

Lactic acid bacteria are often regarded as preservative organisms in VP meats and they generally do not cause noticeable organoleptic spoilage until long after maximum populations are reached (Egan and Shay, 1982; Borch and Nerbrink, 1989). The end-products of LAB metabolism under anaerobic conditions vary depending primarily on pH of the meat and its glucose concentration (Thomas et al., 1979; Rhee and Pack, 1980; Borch and Molin, 1989). The type of end-products produced determines if VP fresh meats will spoil early or after maximum population has been reached.

After prolonged anaerobic storage, fresh meat is often described as having a "sour" or "acid" off-odour. This souring has been attributed to the production of acetate (Sutherland et al., 1976), methanethiol and dimethyl sulfide (Edwards and Dainty, 1987), and short chain organic acids (Sutherland et al., 1976; Dainty, 1981). With a source of unlimited carbohydrates, homofermentative LAB produce lactate as the sole product of anaerobic fermentation. However, under conditions of limiting glucose, which occurs after maximum population is reached, homofermentative LAB can produce ethanol, acetate, formate, and CO_2 via the formate lyase pathway (Condon, 1987; Hjörleifsdottir et al., 1990; Borch et al., 1991; Borch and Agerhem, 1992). Under anaerobic conditions heterofermentative *Leuconostoc* spp. produce lactate, ethanol, and CO_2 from glucose fermentation; but under aerobic conditions the production of acetate is favored over ethanol (Condon, 1987). On meat stored anaerobically, a *Leuconostoc* sp. produced ethanol and a *Lactobacillus* sp. produced acetate (Borch and Agerhem, 1992). Under strict anaerobic conditions acetate production is not favored by *Leuconostoc* spp.;

however, commercial, high oxygen barrier bags have some level of oxygen permeability, thus acetate production could occur. Research has shown that LAB account for noticeable increases in acetic acid in meat (Dainty, 1981; Borch and Agerhem, 1992).

Sharp (1979) suggested that metabolic end-products could be used to accurately measure food quality, instead of enumerating bacterial numbers. In particular, D-lactate has been used to measure spoilage of VP meats (Sinell and Lücke, 1979; de Pablo et al., 1989; Borch and Agerhem, 1992). More research is required to determine the feasibility of using LAB end-products as a tool for objective measurement of spoilage, primarily because not all LAB produce the same end-products.

Certain LAB are potent spoilage organisms that can cause early spoilage of VP meats, before maximum population is reached. Under conditions of glucose limitation, as with DFD meats, amino acids will be metabolized. Cysteine is degraded to produce H_2S, which imparts an offensive sulphur odour to meats. Many researchers have reported the detection of sulphur odours in meat when LAB are present (Shay and Egan, 1981; Hanna et al., 1983; Egan et al., 1986; Edwards and Dainty, 1987; Schillinger and Lücke, 1987a). Hydrogen sulfide producing strains of lactobacilli have been identified as *Lactobacillus sake* (Shay and Egan, 1981; Kandler et al., 1988). Hydrogen sulfide production has been linked to the undesirable greening that is occasionally observed in VP meats. Greening is due to the formation of sulfmyoglobin (Egan et al., 1989), and is usually associated with high pH meats (Nicol et al., 1970; Taylor and Shaw, 1977; Gill and Newton, 1979; Egan et al., 1986).

Spoilage of fresh meats by LAB can occur via transamination, decarboxylation, and reduction reactions that have been linked to the production of aldehydes and ketones in fresh meats (Dainty and Mackey, 1992). Acetate, which has been linked to the souring of VP meat, may be produced by deamination of alanine, cysteine and serine. Edwards et al. (1987) concluded that decarboxylation of amino acids increased the concentration of

the diamines cadaverine, putrescine, and tyramine, during vacuum storage of fresh, chilled meats. Cadaverine and putrescine, which have obnoxious odours, are produced by some *Enterobacteriaceae* in VP beef (Dainty et al., 1986). Zee et al. (1981) discovered that putrescine could be produced by *Lactobacillus brevis*, while Dainty et al. (1986) found that *Enterobacteriaceae* could decarboxylate ornithine, produced by LAB, to yield putrescine. Thus, LAB can contribute to the spoilage of fresh VP meats due to diamine production.

Volatile compounds present in the storage environment of anaerobically packaged meats may provide an objective tool for spoilage measurement. Edwards and Dainty (1987) studied the volatile compounds present in high and low pH VP pork. Normal pH pork had a dominant LAB population after 5°C vacuum storage and the volatile compounds detected were hydrogen sulfide, dimethyl disulfide, methanethiol, methylthioacetate, dimethyldisulfide and 3-methylbutanol. The odour of the meat was described as "sour". In contrast, gram-negative organisms were the most prevalent on high pH pork and resulted in the production of additional sulphur compounds and short chain fatty acids, resulting in an obnoxious off-odour. The pH of fresh VP pork is vital for determining the eventual storage life, because high pH pork (6.2 to 6.5) stored at 5°C spoils in 2 to 3 weeks with noticeable greening and sulphur odours; normal pH pork (5.4 to 5.8) spoils in 3 to 4 weeks due to souring (Egan et al., 1986).

The effects of individual strains of LAB on the sensory quality of fresh, anaerobically stored meats has been an important focus of research. Sterile meat inoculated with pure cultures of LAB has been used to observe the spoilage pattern of the organism in question. Smith et al. (1980) inoculated sterile beef steaks with pure cultures of lactobacilli and found that uninoculated control steaks showed less off-odours, discoloration, and better flavour. Pure cultures of LAB added to VP beef resulted in increased rates of spoilage due to the detection of sour, acid, or bitter flavours after

bacterial populations exceeded 10^8 LAB/cm^2 (Egan and Shay, 1982). However, these researchers reported that the unninoculated sterile controls spoiled after 28 days of vacuum storage. These findings are intriguing because it appears that sterile VP meat eventually spoils due to nonmicrobial changes. Two homofermentative *Lactobacillus* spp. and a *Leuconostoc* sp. were found to spoil beef slices at similar rates in terms of flavour, but the *Leuconostoc* strain caused noticeable off-odours (Egan and Shay, 1982). In contrast, Borch and Agerhem (1992) reported that *Leuconostoc* spp. caused more rapid flavour changes than *Lactobacillus* spp. Leisner et al. (1995) showed that two strains of *Carnobacterium* produced off-odours in VP beef after 8 weeks of vacuum storage at 2°C. Inoculation of sterile meat with pure cultures of LAB to study the spoilage patterns of individual organisms has been criticized. It is argued that meat spoilage depends on a number of factors and that organisms behave differently and may contribute to the spoilage process in different ways, depending on interactions with the other microorganisms present.

Clearly, the spoilage of fresh VP meat encompasses a number of interrelated factors. Implicating individual organisms or intrinsic chemical changes within the meat itself as the reason or reasons for spoilage is extremely difficult, because spoilage appears to be associated with to an array of phenomena. From this review, it is clear that LAB are capable of spoiling VP meats. Generally, spoilage due to LAB occurs after maximum population is reached, resulting in an extension of storage life for chilled, VP meats. However, LAB can cause early spoilage in VP meats if a sulphur producing strain grows under conditions of limiting glucose and appropriate oxygen concentrations (Egan et al., 1989).

The dominance of LAB during extended storage of anaerobically packaged meat is well known. However, bacterial growth and spoilage of meat upon transition from anaerobic to aerobic retail display environments is not well documented. Greer et al.

(1993) reported that LAB remain the dominant group of bacteria during 0 to 9 days of retail display at 8°C on pork loin chops prepared from anaerobically packaged loins. These results could have important practical significance if the domination of LAB provides an inhibitory effect on other adventitious bacteria during aerobic retail display. These researchers reported a negative correlation between time of anaerobic pork loin storage and retail case life of chops cut from pork loins. Prolonged anaerobic storage of pork loins compromises the aerobic display life of chops.

1.5. RAPID CHILLING OF VACUUM PACKAGED MEATS

Accelerated processing of fresh meat is attractive to meat industry management because of numerous advantages over conventional processing that ultimately lead to increased profitability. These advantages include reduced in-plant holding time, lower cooler space requirements, decreased energy requirements (Wiley et al., 1989), faster throughput, and decreased evaporative losses (Joseph, 1996). In order to achieve accelerated processing, carcasses must be rapidly chilled. There are numerous reports pertaining to the rapid chilling of carcasses (Mandigo et al., 1979; Swasdee et al., 1983; Crenwelge et al., 1984; Gigiel and James., 1984; Gariépy et al., 1995; Joseph, 1996); however, very little research has been carried out to determine the microbiological and quality effects of rapid chilling of VP fresh meats.

The conventional procedure after slaughter and dressing is to move carcasses into a storage cooler immediately after blast chilling at air temperatures of -25°C to -40°C for 40 to 60 minutes. For pork processing, carcasses are usually held in the storage cooler (2 °C) for approximately 24 hours, at which time they are fabricated into primal and sub-primal cuts, VP, boxed, and moved into a shipping cooler. To achieve optimum storage life, muscle tissue should be chilled to -1°C immediately after carcass dressing, and remain at -1°C throughout the fabrication and packaging process and boxed, packaged

meat should be held at -1.5°C for distribution. This temperature regime is impossible to achieve under commercial conditions. Assuming an abattoir operates with Good Manufacturing Practice from slaughter to packaging, a maximum storage life will be achieved only when packaged product entering the shipping cooler or refrigerated truck is at or below the air temperatures maintained in these two areas. Traditionally, freshly boxed VP product has a slightly higher temperature than the air temperature of the shipping cooler and refrigerated truck trailer, which are usually kept between -1°C and 1°C. Rapid chilling immediately after VP can result in product with a surface temperature at or below the air temperature of the shipping cooler or truck trailer. Rapid chilling is particularly important when the packaging process used exposes the VP product to a heat shrink, which significantly warms the meat surface prior to boxing. Ultimately, the goal is to temper the entire product, not just the surface, to the air temperature of the shipping cooler or truck trailer. However, in today's high volume, fast throughput industry, this is impossible due to time constraints. Frye et al. (1985) showed that at least 80 minutes was needed to chill hot boned boneless pork loins to an internal temperature of -2°C using rapid chilling techniques. There have been no reports of how long it takes to chill primal cuts from an internal temperature of 2°C, a realistic internal temperature immediately prior to packaging, to -1°C utilizing rapid chilling or other techniques. This would be extremely useful research for the meat industry because if reducing the internal temperature of primal cuts to -1°C is feasible, product would be boxed at the optimum of -1°C resulting in maximum possible storage life.

Rapid chilling of hot boned pork loins with -20°C propylene glycol or -30°C blast chilling, resulting in a crust freeze, has no beneficial effects in terms of microbiology and palatability compared with hot boned pork loins that are conventionally chilled (2°C) (Weakley et al., 1986). Hot boned pork loins that are chilled rapidly had higher bacterial counts and higher levels of off-odours after vacuum storage compared with

conventionally chilled VP pork loins (Weakley et al., 1986). In contrast, hot boned ground beef chilled rapidly with CO_2 snow or -2°C brine had lower microbial numbers compared with conventionally processed ground beef after vacuum storage (Abu-Bakar et al., 1988). Wiley et al. (1989) evaluated the effects of rapid chilling of hot boned pork loins with -3.3°C brine solution and a CO_2 chill tunnel (-79°C). The two rapid chilling treatments had no effect on sensory traits after vacuum storage; no microbiological analysis was done. No known studies that attempt to evaluate the effects of commercial, rapid chilling on bacteriology and storage life of conventionally produced primal cuts have been published.

1.6. PREDICTING BACTERIAL GROWTH WITH TEMPERATURE FUNCTION INTEGRATION

Evaluation of the microbiological consequences of the temperatures experienced by fresh meats during storage and distribution can be exceedingly difficult. Microbial sampling must be done at the conclusion of the storage or distribution process being evaluated. This is difficult under commercial circumstances for two reasons. First, fresh product must be sacrificed in order to collect a significant amount of microbiological data. Second, in instances where fresh meat is being shipped to distant markets, it is impossible to carry out microbial analyses, as product is no longer accessible. A method has been developed that predicts microbial growth during storage or distribution without microbial sampling. This method is known as temperature function integration. Temperature function integration is based on mathematical models that have been developed for individual organisms. Each bacterium possesses its own minimum, maximum, and optimum growth temperatures. Models pertaining to the growth of specific organisms have been developed by studying their growth rates in laboratory media or food products (Gill, 1996). The data collected are then plotted as the square

root of the growth rate against the temperature (Ratkowsky et al., 1982; McMeekin et al., 1988). From the regression line fitted to the data, equations or models describing the growth rates of each microorganism at different temperatures are formed (Gill et al., 1995). These models are used to predict the growth of the organism during each of the recorded intervals, with the growth during each individual interval being summed to arrive at the total growth during the storage or distribution process (Gill et al., 1995).

Product temperature histories are collected with portable temperature data loggers that can easily be placed inside boxes of fresh meat destined for storage or distribution (Gill and Jones, 1992). These data loggers record the temperatures experienced by the product and the temperature history data is then integrated with respect to models relating temperature and growth rate for various microorganisms (Gill, 1986). Integration is carried out by a computer program that performs the necessary calculations and provides the user with a printout containing the predicted growth, in numbers of generations of various bacteria for the recorded temperature history.

Initially, a linear curve developed by Spencer and Baines (1964) was used to predict spoilage of fresh fish. However, Olley and Ratkowsky (1973) developed a more appropriate model based on the Arrhenius equation that can predict rates of microbial spoilage of flesh foods. Daud et al. (1978) applied this equation to predict accurately the shelf life of poultry tissue at storage temperatures up to 16°C. Over the past decade, temperature function integration has primarily been used to evaluate the hygienic efficiency of various commercial beef chilling, storage, and distribution processes. The model pertaining to aerobic growth of *Escherichia coli* was used for these evaluations, because *E. coli* is a suitable indicator of the behavior of enteric pathogens on carcass surfaces (Gill, 1986). Gill (1986) reported that temperature function integration could provide a simple method to evaluate the bacteriological consequences of the cooling of offals. The growth of *E. coli* predicted by calculation varied less than one generation

from growth parameters derived by traditional plating methods. Temperature function integration provides meat industry management with a tool that allows them to focus on and improve parts of offal production that are vital in assuring adequate hygiene of the final product (Gill and Jones, 1992a).

Temperature function integration has similarly been used to observe the hygienic adequacy of chilling processes of commercial beef carcasses (Gill et al., 1991a,b). It was shown that the warmest location of the carcass (the aitch-bone pocket) had the highest estimated *E. coli* growth during the 24 hour chilling process and the time for the beef sides to chill to a deep temperature of 7°C conformed with Good Manufacturing Practices (Gill et al., 1991a). Gill et al. (1991b) reported that a spray chilling process for beef carcasses showed similar hygienic characteristics compared with a conventional process. Reichel et al. (1991) reported that a hot boning process for beef was hygienically inferior to conventional processing; however, with process improvement, hot boning of beef had similar calculated *E. coli* proliferation to that predicted for conventional processing.

Temperature function integration has recently been used to predict the storage efficiency of various commercial storage and distribution processes. A storage efficiency factor can be calculated from the estimated proliferation of the appropriate organism by a computer program; this value is then compared with the estimated growth of that organism under optimum storage conditions (-1.5°C). The storage efficiency is the percentage ratio of the growth of the organism(s) in question calculated for a product temperature of -1.5°C throughout transportation and the growth of the organism(s) in question predicted from the product temperature history (Gill and Jones, 1992b; Gill and Phillips, 1993). The appropriate model to be used in different situations depends on the intrinsic and extrinsic conditions of the product in question and which organisms will dominate the spoilage process under those particular conditions.

For VP meat with a high pH, psychrotrophic *Enterobacteriaceae* can be important in the spoilage process (Egan and Shay, 1984; Egan et al., 1986; Gill and Greer, 1993). The model for anaerobic growth of psychrotrophic *Enterobacteriaceae* was utilized to calculate the storage efficiency of a commercial process for distribution of boxed, VP beef in refrigerated truck trailers (Gill and Jones, 1992b). The storage efficiency varied from 18.8% to 33.9% depending on the position of the meat in the trailer. Boxes near the rear of the trailer had higher storage efficiency values than boxes in other positions in the trailer. The low storage efficiency values were obtained because the product temperature histories were much higher than the optimum (-1.5°C; Gill et al., 1988a) in almost every instance. This optimum storage temperature is almost never attained for product being shipped within North America (Gill and Jones, 1992b).

For primary storage or transport processes with anaerobically packaged products, calculations should include any lag phase that is induced due to the shift from aerobic to anaerobic conditions (Gill and Phillips, 1993). A model for the lag phase induced for facultatively anaerobic, psychrotrophic *Enterobacteriaceae* by shifting from aerobic to anaerobic conditions was developed (Gill and Jones, 1992b). However, secondary transport processes after a short storage period are evaluated with temperature function integration assuming the worst case scenario, i.e., growth of the organism in question commenced at the beginning of the transport process (Gill and Jones, 1992b).

Evaluation of storage or transport processes where meat is stored aerobically is carried out using a model that predicts the growth of *Pseudomonas* spp. Beef sides shipped by rail showed storage efficiencies in excess of 55%, while sides shipped by refrigerated truck had storage efficiencies less than 55% (Gill and Phillips, 1993).

When calculating storage efficiencies for anaerobically packaged, normal pH meats, a model that is indicative of LAB growth is used. A model based on the growth of *Leuconostoc* spp. was first used by Gill et al. (1995) to calculate the storage efficiency

during the shipment of wiener sausages by refrigerated truck. The researchers used other models to calculate the growth of *E. coli* and the psychrotrophic pathogen *Listeria monocytogenes*. In most cases no growth of *E. coli* was reported; however, growth of *L. monocytogenes* was reported in all instances.

Temperature function integration has drawn criticism from other researchers. It is argued that meat systems are complex in terms of their microbial composition, intrinsic factors, nonmicrobial deterioration and interactions between organisms; consequently, they argue that microbial growth and storage life cannot be predicted using mathematical equations (McMeekin and Ross, 1996). However, when microbial sampling is difficult or impossible, temperature function integration provides an objective means of assessing and comparing processes and it enables researchers to quantify the effects of changing temperatures on microbial growth (Gill, 1996). From models, a processor or researcher can determine how close a particular process is operating to the theoretical optimum. Even with its drawbacks, temperature function integration can provide valuable information to the fresh meat industry.

1.7. OBJECTIVES OF RESEARCH

Traditionally, Alberta pork producers have been shipping significant quantities of frozen pork to distant export markets. However, Alberta pork producers have recently increased the amount of fresh, unfrozen pork shipped to distant markets. This has been the result of greater consumer acceptance and higher economic returns from the fresh product. For Alberta pork producers to emerge as one of the dominant exporters of fresh pork, a consistent, high quality product must be produced. Fresh, chilled pork produced for export markets must have minimal purge and it must have a guaranteed storage life.

Currently, there is very little information pertaining to the effects on the quality and storage life of fresh pork of technologically advanced packaging systems used by Alberta pork producers.

The objectives of this study were to:

1. evaluate the current packaging process utilized by an Alberta pork packing plant in terms of time/temperature parameters of the packaging system (Chapter 2).
2. evaluate the effect of the current packaging process on bacteriology, storage life, purge accumulation, and retail case life of VP pork loins and butts (Chapter 3).
3. evaluate the sensory consequences of the current packaging process on VP pork loins and butts (Chapter 4).
4. determine if the rapid chilling portion of the current packaging process is required to prepare VP, boxed meat for overseas shipment (Chapter 5).

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2. Characterization of a Commercial Pork Packaging and Shipping Process for Export to Japanese Markets

2.1 INTRODUCTION

The development of preservative packaging has revolutionized the fresh meat industry. Anaerobic packaging, under vacuum, has dramatically increased the storage potential of fresh, chilled meats by inhibiting the growth of putrefactive bacteria and selecting for the growth of nonputrefactive lactic acid bacteria (LAB). Anaerobic spoilage of fresh, chilled meats by LAB usually occurs some time after maximum population has been reached, resulting in an extension of storage life. Fresh, chilled pork, stored under vacuum can remain fresh for weeks longer than is possible with traditional, aerobic packaging (Smith et al., 1980; Seideman et al., 1980; Lee et al., 1985; Shay and Egan, 1987). This extension of storage life has enabled the North American meat industry to take advantage of lucrative, distant, export markets. In particular, Alberta pork producers have been able to reach distant markets, such as Japan, with fresh, unfrozen product. Until recently Alberta pork producers shipped significant amounts of frozen product to Japan, where shipping time can exceed 14 days. Fresh, unfrozen product that is packaged aerobically would spoil during this lengthy shipping time. As a consequence, Alberta pork producers welcomed vacuum packaging technology because the extended storage life has allowed them to change from frozen product to chilled, unfrozen product for shipment to Japan. Increasing the volume of fresh, unfrozen product shipped to Japan has allowed Alberta pork producers to reap the rewards of shipping unfrozen product; most notably, greater consumer acceptance and higher economic returns.

Currently, a number of Alberta pork producers have adopted a technologically advanced packaging system developed by the Cryovac Division of W.R. Grace & Co.

This system vacuum packages product in a high oxygen barrier package, and then exposes the packaged product to heat, which shrinks the package around the product. The packaged product is then conveyed through a CO_2 chill tunnel. Product is then boxed for shipment to local and export markets. A pork abattoir based in Red Deer, Alberta is currently utilizing this technologically advanced packaging system to package fresh pork destined for Japan. A number of studies have been undertaken that focus on the evaluation of various commercial meat production processes (Gill et al., 1991a; Reichel et al., 1991; Gill et al., 1991b; Gill and Jones, 1992; Gill and Phillips, 1993; Gill et al., 1995); However, to date, there has been little documentation of the temperatures achieved in fresh pork during production and shipment to Japan. It is well established that the rate of microbial growth and metabolism are highly temperature dependent, and thus, the storage life of vacuum packaged meat is also temperature dependent (Zamora and Zaritzky, 1985). Consequently, the microbial quality and, in turn, the storage life of fresh pork arriving in Japan depends on the temperature regime experienced during packaging, storage, and shipment. As global competition increases Alberta pork producers must produce a high quality product with a guaranteed storage life if they are to emerge as one of the dominant exporters of fresh pork to distant markets.

The objective of this study was to determine the time/temperature parameters experienced by fresh pork loins during packaging with the Cryovac packaging system, during short term storage prior to shipment, and during shipment from Red Deer to Japan.

2.2 MATERIALS AND METHODS

2.2.1 Pork production process

The study was done at a modern pork abattoir that slaughters 4500 hogs per day. Following dressing, pork carcasses were spray chilled with cold water, blast air chilled at -30°C for 50 minutes, then subjected to a second spray chill before storage in a cooler

where the air temperature was maintained between 2 and 3°C. For this study carcasses were held overnight, however, carcasses are held longer if chilled before nonworking days. Following the holding period, carcasses were fabricated into primal cuts. Fabrication for each carcass takes approximately 40 minutes. The primal cuts were vacuum packaged, conveyed through a heat shrink, and then through a CO_2 chill tunnel, which operates at approximately -80°C, and is 13.7 m long by 1.3 m wide. As product exits the CO_2 chill tunnel it was boxed immediately. Filled boxes were manually put through an automatic sealer and were placed on pallets in a shipping cooler that was operated at an air temperature between -1 and 1°C. Boxes of fresh pork loins were usually held in the cooler for 1 to 8 hours on a normal production day. Following the holding period boxes were removed from pallets and stacked in truck trailer containers for road dispatch to Vancouver. The gap between stacked boxes and the trailer ceiling was about 1 m. The amount of boxed meat packed into truck trailers depended on the production day, but trailers are never filled to more than half of the available trailer volume. The truck trailer refrigeration unit was switched on when the trailer loading was approximately 1/3 complete, and it was set to deliver an air temperature of -1.1°C. Once loaded, trailers were sealed and shipped to Vancouver. In Vancouver the trailer was transferred to a seagoing vessel and shipped to Japan. Transport to Japan takes 11 to 13 days, depending on which Japanese port of entry. Trailers were not opened at any point during road or sea transport. Figure 2.1 shows a flow chart of the packaging and shipping process for pork loins destined for Japan from an abattoir in Red Deer, Alberta.

HOGS ARE SLAUGHTERED

⇓

CHILLED OVERNIGHT

⇓

FABRICATION

⇓

VACUUM PACKAGED

⇓

HEAT SHRINK OF PACKAGE

⇓

CO_2 CHILL TUNNEL

⇓

BOXED FOR SHIPMENT

⇓

SHIPMENT TO VANCOUVER

⇓

SHIPMENT TO JAPAN

Figure 2.1. Flow chart of process for the shipment of fresh pork loins to Japan from an abattoir in Red Deer, Alberta.

2.2.2. Product

All temperature measurements were made on fresh 2 to 3 kg *longissimus dorsi* (loin) muscles with a commercial subcutaneous fat trim (5 mm). For portions of the study that involved boxed product, six pieces were placed in each box, which is consistent with commercial practice.

2.2.3. Collection of time/temperature data for the packaging process

Temperature data was recorded with a Physitemp digital thermometer (model BAT-12; Physitemp Instruments Inc., Clifton, NJ) fitted with an external temperature probe (type T-model 8528-23; Cole-Parmer, Chicago, IL). Time data was collected with a hand held timer. Immediately after fabrication, surface and internal temperatures of fresh loins were recorded. Internal temperatures were recorded by inserting the tip of the external temperature probe to the approximate center of the loin; surface temperatures were recorded by inserting the tip of the external temperature probe into the first few millimeters of muscle tissue. Over a period of 7 months, 61 surface and 61 internal temperatures were collected. When loins exited the heat shrink, surface temperatures were recorded by placing the external temperature probe between two loins. Over the course of 7 months, 71 temperatures were recorded. At the entrance to the CO_2 chill tunnel, a total of 56 surface temperatures were recorded, as described for loins exiting the heat shrink. The surface temperature of 90 loins exiting the CO_2 chill tunnel were recorded and the internal temperature of 23 loins was determined immediately after they exited the CO_2 chill tunnel. The time required to convey loins from the heat shrink to the entrance of the CO_2 chill tunnel and for passage through the CO_2 chill tunnel were recorded, 64 and 110 times, respectively, over the 7 month testing period.

2.2.4. Collection of product temperature histories

Product temperature histories were collected using MIRINZ-Delphi temperature data loggers (Tru-Test, Auckland, New Zealand.). The loggers operate in the range of -20 to 40°C with an accuracy of ± 0.25°C and a resolution of 0.25°C. To examine the effects of the CO_2 chill tunnel on the temperature of boxed product during storage in the shipping cooler, six vacuum packaged loins were conveyed through the heat shrink and the CO_2 chill tunnel before boxing, while another six were boxed immediately after exiting the heat shrink. The two boxes were placed on a shelf in the shipping cooler. Product temperature histories were recorded at the center position of the box, in accordance with the position proposed by Gill and Jones (1992) for recording product temperature histories of boxed product that is cooling during storage. This experiment was repeated 7 times over a 7 month period, with storage time in the shipping cooler ranging from 1 to 4 days. After the storage period, temperature data loggers were removed from boxes and the temperature data were downloaded to a computer to obtain product temperature histories.

For recording product temperature histories during transport of boxed, vacuum packaged loins from Red Deer to Japan, temperature data loggers were placed at the central position of boxes containing loins prior to placing boxes on a pallet in the shipping cooler. The boxes containing loins and a data logger were shipped to Japan with regular weekly shipments. Upon arrival in Japan data loggers were recovered by customers and returned to Red Deer by courier. A total of six boxes of loins containing data loggers were shipped to Japan (3 trials; 2 boxes/trial). Data loggers for trial #2 were lost during transit, thus only four temperature histories were collected for product shipped to Japan. Two temperature histories were recorded at random locations in a refrigerated trailer, one temperature history was recorded near the rear of the trailer, and one was recorded at a central position in the refrigerated trailer.

2.3. RESULTS

2.3.1. Time/temperature measurements for the packaging process

The data in Table 2.1 show the surface and internal temperatures of fresh pork loins at various points throughout the packaging process. The mean internal temperature recorded for fresh pork loins prior to vacuum packaging was 2.9°C, which suggests that the carcass chilling process successfully reduced the temperature of the loin section of the carcass prior to fabrication. As the product moved through the packaging process, and in particular as the product exited the heat shrink tunnel, the surface temperature of fresh pork loins was dramatically higher than the surface temperatures recorded prior to vacuum packaging. In fact, the heat shrink warmed the surface of fresh pork loins to temperatures as high as 21.2°C. This could be considered an extremely undesirable surface temperature if product was boxed at this stage. Immediately after the loins exited the heat shrink tunnel, the surface temperature fell; however, the mean surface temperature of 16.1°C prior to entering the chill tunnel would still be considered unacceptably warm for boxing. Vacuum packaged pork loins exited the CO_2 chill tunnel with a surface temperature ranging from -0.8 to -1.6°C, which is markedly lower than the surface temperature of the pork loins prior to entering the CO_2 chill tunnel. It is important to note that the internal temperature of fresh pork loins packaged with the Cryovac packaging process was not affected by the overall packaging process, including the CO_2 chill tunnel. The mean internal temperature of fresh pork loins was 2.9°C prior to vacuum packaging and after exiting the CO_2 chill tunnel. The time required for pork loins to pass through the CO_2 chill tunnel ranged from 2 minutes 19 seconds to 4 minutes 33 seconds, mean 3 minutes 9 seconds, standard deviation ± 42 seconds. The CO_2 chill tunnel successfully removed heat from the surface of fresh, vacuum packaged pork loins after exposure to heat shrink and lowered the surface temperature to near the optimum storage temperature of -1.5°C prior to boxing and shipment of the product.

Table 2.1. Internal and surface temperatures of fresh pork loins at various points during the packaging process.

POINT IN PROCESS	Surface Temperature (°C)				Internal Temperature (°C)			
	MIN.	MAX.	MEAN	STD. DEV.	MIN.	MAX.	MEAN	STD. DEV.
Prior to Vacuum Packaging	4.5	5.2	4.8[1]	0.14	2.5	3.3	2.9[5]	0.19
After Exiting Heat Shrink	15.6	21.2	18.5[2]	1.5	-	-	-	-
Prior to Entering CO_2 Chill Tunnel	14.0	18.9	16.1[3]	1.4	-	-	-	-
After Exiting CO_2 Chill Tunnel	-1.6	-0.8	-1.2[4]	0.25	2.4	3.4	2.9[6]	0.27

-not determined.
[1] Mean of 61 measurements.
[2] Mean of 71 measurements.
[3] Mean of 56 measurements.
[4] Mean of 90 measurements.
[5] Mean of 61 measurements.
[6] Mean of 23 measurements.

2.3.2. Product temperature in the shipping cooler

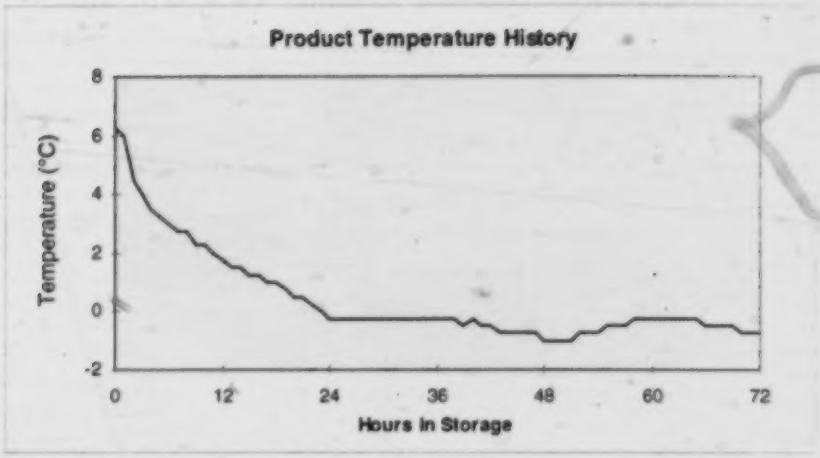
Temperature histories were collected from 14 boxes of pork loins stored in the shipping cooler. Table 2.2 shows the mean surface temperatures at various times during storage in the shipping cooler for boxes that contained loins exposed to the CO_2 chill tunnel and for boxes that contained loins that were not exposed to the CO_2 chill tunnel. Initially the mean surface temperature of boxed pork loins that were exposed to the CO_2 chill tunnel were 6.8°C cooler than the mean surface temperature of boxed loins that were not exposed to the CO_2 chill tunnel. After 8 hours of storage the mean surface temperature of both the loins that had passed through the chill tunnel and those that had not passed through the chill tunnel warmed to 0.6°C and 2.8°C, respectively. However, the difference in mean surface temperature between 'chill' and 'no chill' loins decreased to 2.2°C. After 24 hours of storage in the shipping cooler the mean surface temperatures of 'chill' and 'no chill' loins were similar for all 7 trials. For the 'no chill' loins the mean initial surface temperature and the mean maximum surface temperature during storage were identical; however, there was a difference of almost 3°C for the 'chill' loins (Table 2.2). Figure 2.2 shows product temperature histories for boxed pork loins stored in the shipping cooler. Pork loins not conveyed through the CO_2 chill tunnel prior to being boxed took *ca.* 24 hours to cool to near the same surface temperature as their counterparts that were exposed to the CO_2 chill tunnel prior to being boxed. After this convergence of temperature, the two product temperature histories were similar and remained below 0°C throughout the remaining storage time.

Table 2.2. Mean temperatures of boxed pork loins at various times during storage in the shipping cooler at the pork abattoir.

	TEMPERATURE (°C)	
	CHILL TUNNEL	NO CHILL TUNNEL
Initial temperature[1]	-0.5	7.3
Temperature after 8 hours of storage[2]	0.6	2.8
Temperature after 24 hours of storage[3]	-0.7	-0.6
Maximum temperature during storage[4]	2.4	7.3
Minimum temperature during storage[5]	-1.4	-1.4
Average temperature during storage[6]	-0.1	0.6

[1] Data are the means of seven initial temperatures recorded by data loggers.
[2] Data are the means of seven temperatures after 8 hours of storage recorded by data loggers.
[3] Data are the means of seven temperatures after 24 hours of storage recorded by data loggers.
[4] Data are the means of seven maximum temperatures during the entire storage period recorded by data loggers.
[5] Data are the means of seven minimum temperatures during the entire storage period recorded by data loggers.
[6] Data are the means of seven average temperatures during the entire storage period recorded by data loggers.

A)



B)

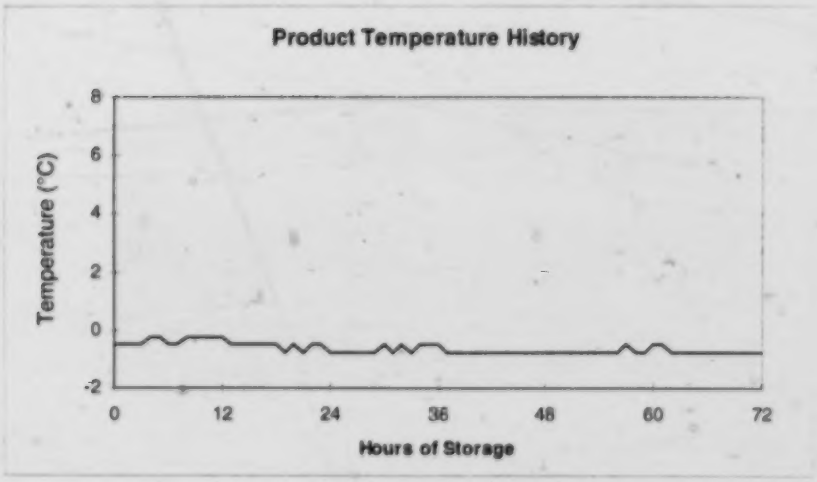


Figure 2.2. Product temperature histories showing the surface temperature of boxed pork loins during storage in the shipping cooler. A) Loins not conveyed through CO_2 chill tunnel prior to boxing. B) Loins conveyed through CO_2 chill tunnel prior to boxing.

2.3.3. Product temperature during shipment to Japan

Four product temperature histories were acquired from boxes of fresh pork loins that were shipped to Japan. Table 2.3 shows the average, maximum, and minimum temperatures for each box as well as the time of travel. The time of travel for boxes #1 and #2 and for boxes #3 and #4 were the same. The average surface temperature of the loins during shipment was below zero and therefore would be considered excellent long distance shipping temperatures. Maximum surface temperatures did not exceed 2.3°C for any of the four boxes. Minimum surface temperatures were at or below -0.5°C for all four boxes and did not drop below -1.0°C for any of the four boxes. Limited fluctuations in surface temperature occurred during transport except for boxes #1 and #4, both of which showed a rise in surface temperature to near 0.5°C after about two days of travel time. The rise in surface temperature for box #4 is shown at point X on Figure 2.3a. The rise in surface temperature for box #1 was similar (data not shown). There was a rise in surface temperature of loins in all four boxes near the end of the journey, shown by point Y in Figure 2.3a and b.

2.4. DISCUSSION

The goal of the fresh meat industry is to package, store, and ship fresh meat in a manner such that the meat experiences the coldest possible temperature regime. Because microbial growth and metabolism are highly dependent on temperature, it is apparent that cold temperature regimes during processing will prolong the storage life and assure safety of meats.

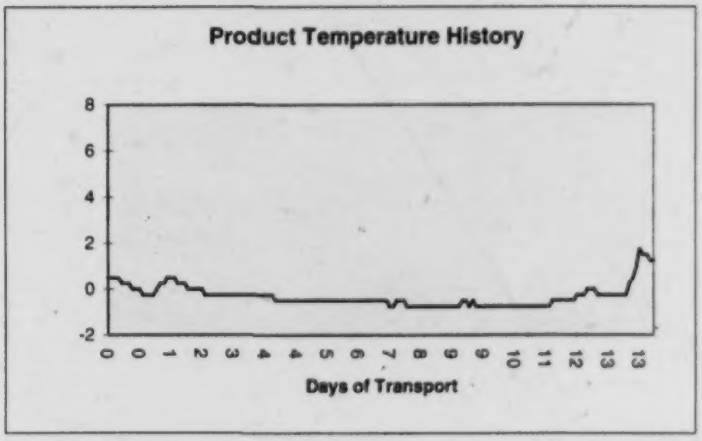
The process characterized in this study resulted in packages of fresh pork loins with surface temperatures that were warmer than the internal temperatures. This discrepancy can be attributed to the fact that the fabricating room was maintained at an air

Table 2.3. Surface temperatures and travel times for boxed pork loins shipped to Japan in a refrigerated trailer.

	TRIAL # (POSITION OF BOX IN TRUCK TRAILER)			
	1 (RANDOM)	2 (RANDOM)	3 (CENTRAL)	4 (REAR)
Time of travel (days)[1]	15.3	15.3	14.3	14.3
Average temperature (°C)[2]	-0.5	-0.3	-0.5	-0.3
Maximum temperature (°C)[3]	1.0	2.3	1.5	1.8
Minimum temperature (°C)[4]	-1.0	-0.5	-0.8	-0.8

[1] Time of travel from the processing plant in Red Deer to arrival in Japan.
[2] Average, [3] maximum, and [4] minimum surface temperatures during travel to Japan.

A)



B)

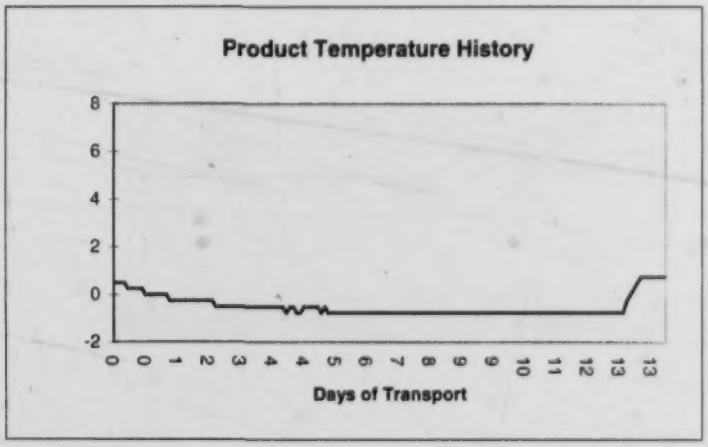


Figure 2.3. Product temperature histories showing the surface temperature of boxed pork loins during shipment to Japan from an abattoir in Red Deer, Alberta: A) temperature logger in a box positioned at rear of refrigerated trailer; B) temperature logger in a box positioned at center of refrigerated trailer.

temperature between 5 and 7°C, which resulted in a surface temperature increase during the 40 minute fabrication process. The heat shrink process further increased the surface temperature of the vacuum packaged loins. However, heat acquired by the surface of packaged pork loins from the heat shrink and from the relatively warm air in the fabricating room was successfully removed by the CO_2 chill tunnel. If fresh, vacuum packaged pork loins are boxed immediately after the heat shrink process, and loaded into refrigerated trailers for shipping, the microbiological consequences could be disastrous, because of the warm temperature regime that fresh, boxed pork loins would experience due to the heat imparted to their surface by the heat shrink, coupled with the substantial insulation effect of the many boxes being packed into refrigerated truck trailers.

It is well documented that muscle tissue starts to freeze at approximately -1°C (Lowry and Gill, 1985; Grau, 1996). The first few millimeters of muscle tissue at the surface of pork loins exiting the CO_2 chill tunnel are frozen, or 'crust frozen'. The belief that freezing of muscle tissue accounts for increased levels of purge in VP meat has raised concern that the CO_2 chill tunnel contributes to the high levels of purge accumulation in VP pork loins shipped from Red Deer to Japan. This concern is investigated in chapters 3 and 4 of this thesis.

To achieve optimum storage life, fresh meat should be chilled to -1°C immediately after carcass dressing, and remain at this temperature throughout the fabrication and packaging process; and it should be stored and shipped in air temperatures of -1.5°C. However, this temperature regime is impossible to achieve due to time and energy constraints, and the fact that plant employees cannot be expected to fabricate carcasses in sub-zero temperatures. From this study it is clear that the Cryovac process, particularly the CO_2 chill tunnel, had no effect on the internal temperature of fresh pork loins destined for Japan. Ideally, boxed pork loins should be held until the product equilibrates to just warmer than -1°C at which time boxes should be loaded into refrigerated truck trailers for shipment to Japan. Unfortunately, this is impossible due to

space, time, and energy constraints. A more realistic and achievable alternative is to reduce the surface temperature of fresh pork loins destined for Japan to near -1°C prior to boxing, short term storage (<8 hours), and shipment. The CO_2 chill tunnel is very important for two reasons: It removes the heat imparted to the surface of fresh pork loins by the heat shrink; and it reduces the surface temperature of fresh pork loins to near the air temperature of the refrigerated truck trailers prior to shipment to Japan. In this regard, coolers and containers are not designed to reduce meat temperatures, but rather to maintain adequate temperature control.

The CO_2 chill tunnel conveyer can be set at various speeds and packaged pork loins should be conveyed through the tunnel at a consistent rate. However, the speed of the conveyer is adjusted according to the volume of product moving through the production line at a particular time. As a result, the time required for loins to pass through the CO_2 chill tunnel had a range of near 2 minutes, which appears to be reality in the commercial situation where the volume of product moving through the production line fluctuates greatly.

Product temperature histories should be recorded at points where product will consistently experience the warmest temperatures (Gill et al., 1991a). Furthermore, because deep muscle tissue is essentially sterile (Gill, 1979), product temperature histories should be recorded at product surfaces, where bacterial contamination and growth occurs. When boxed product is cooling, the warmest meat surfaces will be at the center of the box (Reichel et al., 1991); consequently, product temperature histories should be recorded at this central location (Gill and Jones, 1992). In situations where product has cooled to the temperature of the surrounding air and may experience warming during subsequent storage or shipping, product temperature histories should be recorded at the position between the product and the interior box walls; because this location is likely to be the warmest area (Gill and Jones, 1992).

After fresh pork loins are boxed, the boxes are placed on pallets in the shipping cooler and they are held (<8 hours) before they are loaded into the refrigerated truck trailers. In this study product temperature histories were collected from the surfaces of boxed pork loins that were conveyed through the CO_2 chill tunnel and they were compared with temperatures for product that was not conveyed through the CO_2 chill tunnel. Initial temperatures were dramatically different due to the surface chilling by the CO_2 chill tunnel; however, after 24 hours in storage the surface temperatures of product prepared by either were very similar. Thus, there was a cooling period of approximately 24 hours in which boxed loins that were not conveyed through the CO_2 chill tunnel were approximately same surface temperature as boxed loins that were conveyed through the CO_2 chill tunnel. This similarity in surface temperature after 24 hours of storage can be attributed to the minimal insulation effect when storing two boxes of loins. In this regard, a limited number of boxes were well spaced in the cooler permitting excellent air circulation. It can be hypothesized that if a similar experiment was done with many more boxes of loins (as in a commercial situation) the time required for the surface temperature of product that had not been conveyed through a chill tunnel to cool to the desired storage temperature of -1°C would be greatly extended because of the increased insulation effect due to the larger number of boxes being stacked together and ultimately a larger meat mass. Indeed, this hypothesis was confirmed in Chapter 5 of this thesis.

Product temperature histories recorded during the transport of boxed pork loins to Japan showed that product experiences an adequately low temperature regime during transport. Even though the surface temperature of vacuum packaged loins is substantially lowered to near -1°C by the CO_2 chill tunnel, the internal temperature of the loins remains close to 3°C. Because of this discrepancy between surface and internal temperature at the time of boxing a rise in surface temperature during transport to Japan is expected due to heat flowing from the warm interior portion of loins. This warming phenomenon was reported by Gill and Jones (1992) and was attributed to the warming of product surfaces

by the warmer centers of the meat cuts and it occurred most often with boxes positioned near the center of refrigerated truck trailers where boxes were insulated from the cold circulating air (-1°C). Because of the low average surface temperature of boxed pork loins during shipment to Japan in this study, it can be concluded that the surface temperature cooling provided by the CO_2 chill tunnel combined with the cold (-1.1°C) air temperature in refrigerated truck trailers reduces and almost eliminates the surface temperature warming phenomenon reported by Gill and Jones (1992).

The brief rise in surface temperature that occurred after about 2 days of shipping time on two of the product temperature histories is unexplainable. One of the two product temperature histories that show this rise in surface temperature was recorded in a box of pork loins that was positioned near the rear of the refrigerated trailer during transport to Japan. This lead us to believe that the other product temperature history showing this rise in temperature, which was randomly located in the refrigerated trailer may have also been located at a rear position. The rise in surface temperature for both of these boxes, located in positions where little or no insulation from circulating air would occur, after 2 days of transport may have resulted from opening of the trailer doors when the transport truck arrived in Vancouver. An alternative explanation could be an unexplained increase in the air temperature delivered by the trailer's refrigeration unit, which would have more dramatic warming effect on rear boxes.

The rise in surface temperature of boxed pork loins near the end of the journey to Japan (Fig 2.3) that was observed for all four boxes that were shipped to Japan. This may also correspond to opening of the trailer door when product arrives in Japan. However, this could not be confirmed.

This study has shown that, with exception of brief warming caused by the heat shrink, which was nullified by the CO_2 chill tunnel, the current packaging, brief storage, and shipping processes employed by a Red Deer abattoir for the shipment of fresh,

vacuum packaged pork loins to Japan was in accord with a temperature regime that would be considered acceptable by pork industry.

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3. Effects of a Commercial CO_2 Chilling System on Bacteriology, Storage Life, Retail Case Life, Purge Accumulation, and Sensory Characteristics for Vacuum Packaged Pork

3.1. INTRODUCTION

Vacuum packaging (VP)of fresh pork in bags with a low gas permeability has the potential to greatly extend storage life (Dainty and Mackey, 1992). The anaerobic environment provided by this type of packaging usually prevents the growth of putrefactive *Pseudomonas* spp., which are the major contributors to the aerobic spoilage of fresh pork, and it restricts the growth of *Brochothrix thermosphacta* (Egan et al., 1986). Consequently, the bacterial population of VP pork is usually dominated by nonputrefactive lactic acid bacteria (LAB). The growth of LAB on VP pork results in a slow fermentation process that eventually causes spoilage due to gradual souring (Sutherland et al., 1976; Dainty et al., 1983; Dainty and Mackey, 1992). Souring does not become evident until after maximum populations of LAB has been reached, resulting in the extended storage life. In fact, VP combined with low temperature storage has the potential to extend storage life of fresh pork for weeks past that which is possible with traditional, aerobic packaging (Smith et al., 1974; Seideman et al., 1980; Lee et al., 1985; Shay and Egan, 1987). This extension of storage life has enabled the North American pork industry and, in particular, Alberta pork producers to reach lucrative export markets, such as Japan, where shipping time can exceed 14 days.

A number of Alberta pork producers have attempted to take advantage of the storage life benefits afforded by vacuum packaging and low temperature storage and they have adopted a packaging system developed by the Cryovac Division of W.R. Grace & Co., that combines vacuum packaging with rapid chilling of the packaged product prior to boxing. This technologically advanced packaging system places fresh, unfrozen

product in a high oxygen barrier package, seals the product under vacuum, and exposes the VP product to heat, which shrinks the package around the product. The product is then conveyed through a CO_2 chill tunnel and exits the tunnel with a surface temperature of about -1.5°C, which is the optimum storage temperature of boxed product (Gill et al., 1988a). Muscle tissue freezes at about -1°C (Grau. 1985). Consequently, the first few millimeters of packaged product exiting the chill tunnel are frozen, which is been termed 'crust frozen'.

A number of studies have been undertaken to determine the effectiveness of low temperature storage and vacuum packaging in extending the storage life of fresh pork. However, limited information exists pertaining to the beneficial or detrimental effects of the current packaging systems, involving rapid chilling, utilized by the Alberta pork industry on bacteriology and storage life, in terms of odour, appearance, or flavour of fresh pork. Weakley et al. (1986) showed that rapid chilling, resulting in a 'crust freeze' of pork loins produced by hot processing of carcasses had no beneficial effect on bacteriology, storage life, or sensory characteristics. However, there is little information in the scientific literature on the effects of rapid surface chilling on the quality of conventionally, cold processed primal and subprimal cuts of pork. It has been shown that extended storage of chilled meat in vacuum results in a higher bacterial population and accelerated deterioration of retail acceptability once meat is removed from vacuum for aerobic retail display (Greer and Jones, 1991). However, little information is available in the scientific literature on the effects of the packaging systems used by Alberta pork abattoirs on the retail case life of fresh pork after vacuum storage.

Exudate or purge of VP meat is generally considered to be unattractive and it is associated with low quality fresh meats, especially by consumers in export markets. It is commonly thought that freezing increases the amount of purge (Penny, 1974; Jalong'O et al., 1987; Greer and Murray, 1991); however, it is not clear whether meat that is 'crust frozen' experiences enough tissue damage to cause a significant increase in purge

accumulation in the vacuum package. Increased purge in VP pork shipped to Japan due to the 'crust freezing' caused by the CO_2 chill tunnel may reduce the competitiveness of Alberta pork producers in the Japanese export market, resulting in negative economic impacts for the Alberta pork industry. The Japanese continue to express concern about the volume of purge associated with imported pork.

The present study was undertaken to determine the effects of the current packaging process, particularly the CO_2 chill tunnel, on the bacteriology, storage life, retail case life, and sensory characteristics of fresh,VP pork loins and butts. The effect of the CO_2 chill tunnel on the amount of purge accumulation in the VP primal cuts was also determined.

3.2. MATERIALS AND METHODS

3.2.1. Meat samples

Boneless pork loins and shoulder butts (hereafter referred to as "butts") were obtained from a federally inspected commercial abattoir. Subprimal cuts were selected from normal daily production to acquire a representative sample of normal production and packaging practices. Loins (20) and butts (20) were packaged under commercial conditions with a Cryovac packaging system (W.R. Grace & Co. Cambridge, MA). The Cryovac system places fresh, unfrozen product in high oxygen barrier bags (Cryovac B-650 BAG oxygen transmission rate [OTR] of 30 to 50 cc/m^2/24 h at 1 atm at 25°C). The packaged product is sealed under a vacuum, exposed to heat to shrink the package around the product, and then the packaged product is conveyed through a CO_2 chill tunnel. After packaging, subprimal cuts were boxed, placed on pallets and held in the shipping cooler for approximately 1 hour (CT). Another set of subprimal cuts was packaged as described, except that they were boxed immediately after exiting the heat shrink, and placed on pallets in the shipping cooler where they were held for approximately 0.75

hour (NT). After the brief holding period, the two pallets, were loaded into a refrigerated truck operated at an air temperature of *ca.* 2°C and shipped to Lacombe Research Station (travel time ca. 0.5 hour). Upon arrival the boxed, VP loins and butts were stored for 0, 2, 4, 6, and 8 weeks at either -1°C or 2°C. Two replications of the experiment were done at each storage temperature. The second replicate was done after data collection for the first replicate was complete.

3.2.2. Retail display and case life

After each vacuum storage interval, four loins (two CT and two NT) and four butts (two CT and two NT) were removed from storage and sampled for bacteriological analysis as described in section 3.2.4. After sampling, 10 slices of 0.5 cm thickness were prepared from each loin and each butt with a Globe meat slicer. Prior to the first slicing and between treatments the slicer was washed and rinsed with 60°C water and allowed to air dry for 15 minutes. At each storage interval four additional loins (two CT and two NT) and butts (two CT and two NT) were shipped on ice to the University of Alberta for sensory evaluation (section 3.2.5). The slices from each of the two treatments for each subprimal cut were placed in sterile plastic bins and returned to the cooler (-1°C or 2°C) for no more than 3 hours. Four slices from each bin were overlapped on Styrofoam trays and overwrapped with an oxygen permeable polyvinyl chloride film (Vitafilm Choice Wrap, Goodyear Canada, Inc., Toronto, ON; OTR: 8000 cc/m^2/24 h). Five packages were prepared for each treatment and subprimal cut and all packages were placed at the same latitude in a fan-circulated, horizontal-type, retail case (Model LPM12T, Hill Refrigeration of Canada, Ltd., Barrie, ON). Retail conditions were simulated by illuminating the display case for 12 h/day with incandescent lighting with an intensity of 750 lx at the meat surface. The surface temperature of the slices on retail display was 8°C throughout the study.

On days 0, 2, 5, 7, and 9 of retail display, two slices from each retail package were removed and one slice was re-wrapped individually for assessment of acceptability based on appearance; the other slice was placed in a small plastic vial for assessment of acceptability based on odour. Assessment of retail case life was done by a five member, experienced panel approximately 30 minutes after vials and individual slices were prepared. Odour was assessed using a 5-point acceptability scale (1=acceptable, 5=unacceptable) and appearance was assessed using a 7-point hedonic scale (1=extremely undesirable, 7=extremely desirable). Retail case life was considered to be the time (days) when scores for retail appearance and odour declined to 3.5 (Greer and Murray, 1991). Meat colour was determined using a Minolta Chroma Meter II (Minolta Camera Co., Ramsey, NJ) that measures the reflectance coordinates (L*, a*, b*). After all panelists had evaluated the appearance of the samples, surface pH was measured with a surface electrode with an Oakton microprocessor (model WD-00605-00, Anachemia Scientific, Calgary, AB). Two pH measurements were taken for each slice.

3.2.3. Storage life, pork colour, and pH

Appearance and odour of the vacuum packaged subprimal cuts were evaluated at each storage interval using the same methodology as described for retail assessment. VP samples were evaluated approximately 15 min after the package had been opened. The end of the storage life (in weeks) was considered to be the time when scores for appearance and odour had declined to 3.5. Surface pH of subprimal cuts was measured at six locations (3 lean and 3 fat lateral surfaces).

3.2.4. Bacteriology

At the end of each storage interval 2 of each subprimal cut for each treatment were removed from storage for bacteriological evaluation. Ten cm^2 (5 mm depth) of tissue was aseptically removed from the anterior and posterior ends and from the fat and

lean lateral surfaces of each loin or butt. The four samples for each loin or butt were combined and homogenized for 2 min in 360 ml of sterile 0.1% peptone water using a Colworth Stomacher (Baxter Diagnostics Corp., Canlab division, Edmonton, AB, Canada). Tenfold serial dilutions were prepared using sterile 0.1% peptone water and total psychrotrophic bacteria, presumptive lactic acid bacteria, *Brochothrix thermosphacta* and *Pseudomonas* spp. were enumerated by the spread plate technique and *Enterobacteriaceae* were enumerated by the pour plate technique.

At the end of each retail display time (0, 2, 5, 7, and 9 days), two slices for each treatment were individually, aseptically placed in stomacher bags and homogenized with 100 ml of sterile 0.1% peptone water. The slice used for retail appearance assessment was traced onto aluminum foil and the surface area was determined using a centimeter grid. The average surface area of loin slices in the study was 130 cm^2 and the average surface area of butt slices was 170 cm^2.

Total psychrotrophic bacteria were enumerated on Plate Count agar (PCA; Difco Laboratories Inc., Detroit, MI) after 10 d of aerobic incubation at 4°C. Lactic acid bacteria were enumerated on nonacidified Lactobacilli MRS (MRS; Difco) agar after 5 d of anaerobic incubation at 25°C using a BBL anaerobic system with an atmosphere containing 5 to 10% CO_2 (Becton and Dickenson Co., Cockeysville, MD). *B. thermosphacta* were enumerated on streptomycin sulphate-thallous acetate-actidione agar (STAA; Gardner, 1966) after incubation for 3 d at 25°C. *Pseudomonas* spp. were enumerated on cephaloridine-fucidin-cetrimide agar (CFC: Mead and Adams, 1977) after 2 d at 25°C. *Enterobacteriaceae* were enumerated using overlaid plates of violet red bile agar (Difco) with 1% added Glucose (VRBGA) and after incubation for 18 and 24 h at 35°C. For the bacteriology after vacuum storage the lower limit of sensitivity for the enumeration of *Enterobacteriaceae* was 1.00 log CFU/cm^2 and the lower limit of sensitivity for all other bacteria was 2.00 log CFU/cm^2. For the bacteriology after aerobic retail display for loin slices the lower limit of sensitivity for the enumeration of

Enterobacteriaceae was 0.25 log CFU/cm^2 and 1.25 log CFU/cm^2 for all other bacteria. For the bacteriology after aerobic retail display for butt slices the lower limit of sensitivity for the enumeration of *Enterobacteriaceae* was 0.20 log CFU/cm^2 and 1.20 log CFU/cm^2 for all other bacteria.

3.2.5. Sensory evaluation

Sensory evaluation was done with an 8 to 10 member trained panel. Panelists were selected from a group of faculty members and graduate students and were trained over a five week period. The sensory panel was trained to identify and score the intensity of seven flavour attributes (overall intensity, liver, sour, dairy, metallic, bitter, and other) with a 15 cm line scale anchored with descriptors (none to very strong). The score card used is presented in Appendix 1.

Pork loins and butts were cooked as roasts at 163°C to an internal temperature of 68.5°C. The internal temperature was measured with two copper-constantan thermocouples connected to a Honeywell recording potentiometer. After cooking, roasts were allowed to cool to 60°C and 1.3 X 1.3 cm cube samples were prepared from the internal portion of each roast.

Panel sessions were conducted twice weekly with one subprimal cut evaluated on each day. Evaluation was done in a atmospherically controlled sensory panel room equipped with red lights and eight individual booths. Each panelist was served 6 samples: a fresh reference sample, 4 coded treatment samples and one coded reference sample to serve as a hidden control. The pork cubes were served in small glass jars covered with foil; each jar contained two cubes from adjacent locations of the source roast. Samples were served at ca. 55°C. Each panelist received water and crackers for rinsing and cleansing the palate between samples.

3.2.6. Purge accumulation

On the same day that subprimal cuts were prepared for sensory evaluation the amount of purge accumulation was determined. The subprimal cuts were weighed before and after removal from the package and after excess moisture had dripped from the subprimal surface. Empty packages were dried and weighed. Total purge accumulation was calculated by difference between the total weight and the weight of the subprimal and package and expressed as a percent weight of the original meat sample.

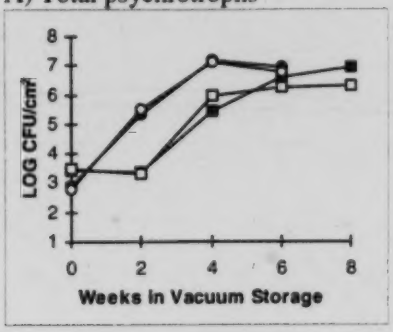
3.3. RESULTS

Fresh pork loins and butts used for this study were acquired from daily production to acquire a representative sample of normal production and packaging procedures. The initial mean pH of fresh pork loins was 5.82 ± 0.04 for the CT loins and 5.88 ± 0.04 for the NT loins. The initial mean pH of the CT butts was 6.14 ± 0.07 and 6.22 ± 0.07 for the NT butts.

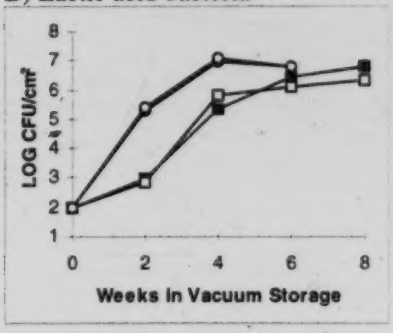
3.3.1. Bacterial growth during vacuum storage

The data in Figure 3.1 shows the effects of the CO_2 chill tunnel on bacterial numbers during storage of VP pork loins at two temperatures. Analysis of variance of data for samples stored at -1°C indicated that the total psychrotrophic ($p<0.001$) and lactic acid bacteria ($p<0.001$) counts for both the CT and NT loins were significantly affected by storage time. However, there was no significant change in the numbers of *Enterobacteriaceae*, *Pseudomonas* spp., and *Brochothrix thermosphacta* on pork loins stored at -1°C ($p>0.05$). Storage at 2°C resulted in a significant increase in the numbers of total psychrotrophs ($p<0.001$), lactic acid bacteria ($p<0.001$), *Enterobacteriaceae* ($p<0.001$), *Pseudomonas* spp. ($p<0.001$), and *B. thermosphacta* ($p<0.05$) on vacuum packaged CT and NT pork loins. The CO_2 chill tunnel did not have a significant effect ($p>0.05$) on the number of the organisms enumerated during vacuum storage at -1°C

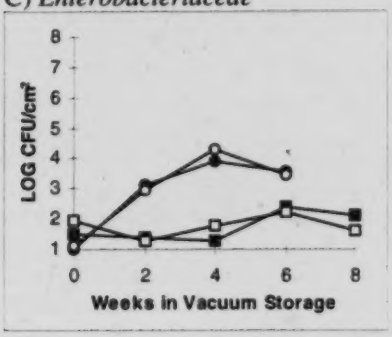
A) Total psychrotrophs



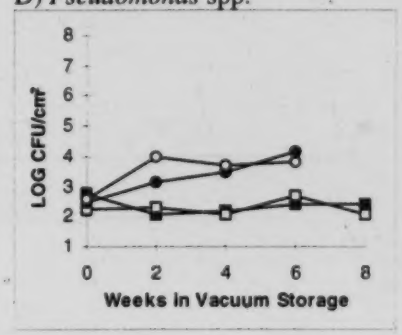
B) Lactic acid bacteria



C) *Enterobacteriaceae*



D) *Pseudomonas* spp.



E) *Brochothrix thermosphacta*

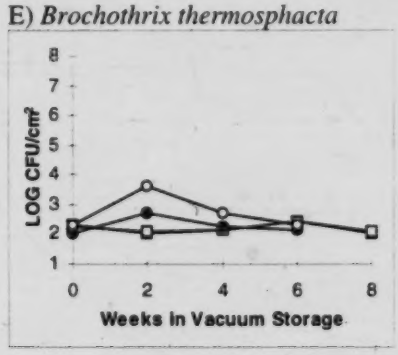


Figure 3.1. Effect of time in vacuum storage at -1°C (□) and 2°C (○) on the growth of A) total psychrotrophs; B) lactic acid bacteria; C) *Enterobacteriaceae*; D) *Pseudomonas* spp.; E) *Brochothrix thermosphacta* on VP fresh pork loins. Solid symbols = VP pork loins that were conveyed through the CO_2 chill tunnel (CT). Open symbols = fresh pork loins that were not conveyed through the CO_2 chill tunnel (NT). Each data point represents the mean of four samples (2 samples for each of 2 replications).

except for total psychrotrophs ($p<0.05$) and *Pseudomonas* spp. ($p<0.05$) after 8 weeks of vacuum storage where counts were slightly higher. At 2°C the CO_2 chill tunnel did not affect ($p>0.05$) the number of the spoilage bacteria except for *Pseudomonas* spp. ($p<0.05$) after two weeks of vacuum storage, where the counts were lower for CT loins.

The data in Figure 3.2 show the effect of the CO_2 chill tunnel on bacterial numbers during vacuum storage of pork butts stored at two temperatures. Increased storage time at -1°C and 2°C significantly increased the numbers of all organisms enumerated ($p<0.001$) on vacuum packaged CT and NT pork butts. The CO_2 chill tunnel did not have a significant effect on the numbers of any of the organisms enumerated during storage at -1°C except for *Pseudomonas* spp. ($p<0.05$) and *B. thermosphacta* ($p<0.05$) after 8 weeks of vacuum storage, where CT butts showed higher counts than NT butts. However, after 4 weeks under vacuum at -1°C all five groups organisms enumerated had higher populations on CT butts compared with NT butts. These differences are likely responsible for higher initial counts on CT butts prepared for aerobic retail display (see section 3.3.3). For 2°C vacuum storage the CO_2 chill tunnel did not have an effect ($p>0.05$) on the growth of any of the spoilage organisms during storage except for *Enterobacteriaceae* ($p<0.05$). After 2 weeks of storage, NT counts were about 1 log higher than CT counts.

Lactic acid bacteria emerged as the dominating bacterial population on all samples after 2 weeks of storage under vacuum. On pork loins, LAB reached a maximum population between 10^6 and 10^7 CFU/cm^2 after 8 weeks of storage at -1°C (Figure 3.1B) and 10^8 CFU/cm^2 after 4 weeks at 2°C. On pork butts, LAB reached a maximum population near 10^8 CFU/cm^2 after 6 weeks of storage under vacuum at -1°C and after 4 weeks of storage under vacuum at 2°C (Figure 3.2B.). Gram-negative bacteria, including *Enterobacteriaceae* and *Pseudomonas* spp. comprised a greater proportion of the bacterial population when samples were stored at 2°C. *Enterobacteriaceae* and *Pseudomonas* spp. both reached populations near 10^4 CFU/cm^2

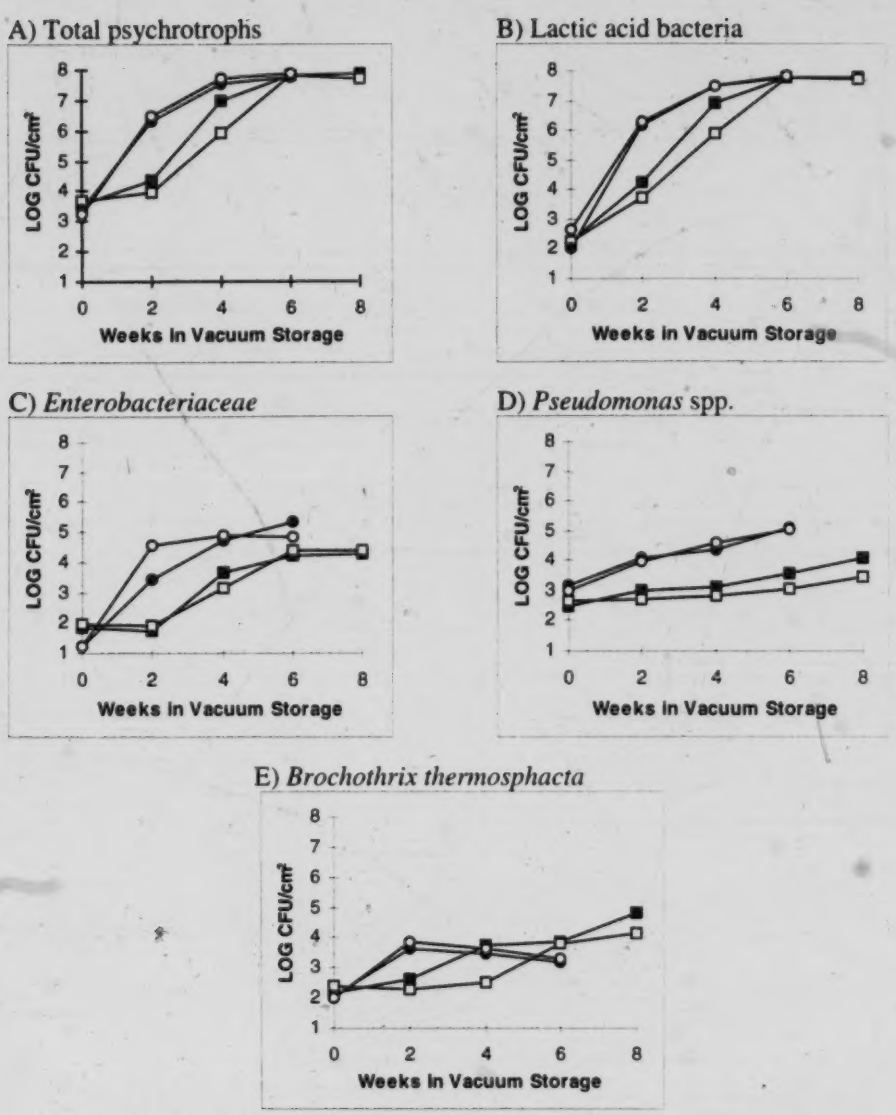


Figure 3.2. Effect of time in vacuum storage at -1°(□) and 2°C (○) on the growth of A) total psychrotrophs; B) lactic acid bacteria; C) *Enterobacteriaceae*; D) *Pseudomonas* spp.; E) *Brochothrix thermosphacta* on VP fresh pork butts. Solid symbols = VP pork butts that were conveyed through the CO_2 chill tunnel. Open symbols = VP pork butts that were not conveyed through the CO_2 chill tunnel. Each data point represents the mean of four samples (2 samples for each of 2 replications).

on pork loins and 10^5 CFU/cm^2 on pork butts when stored at 2°C (Figures 3.1C and 3.2C). During storage at -1°C, *Enterobacteriaceae* and *Pseudomonas* spp. did not grow on pork loins but reached levels near 10^4 CFU/cm^2 on butts (Figure 3.1C,D).

3.2.2. Storage life of VP pork loins and butts

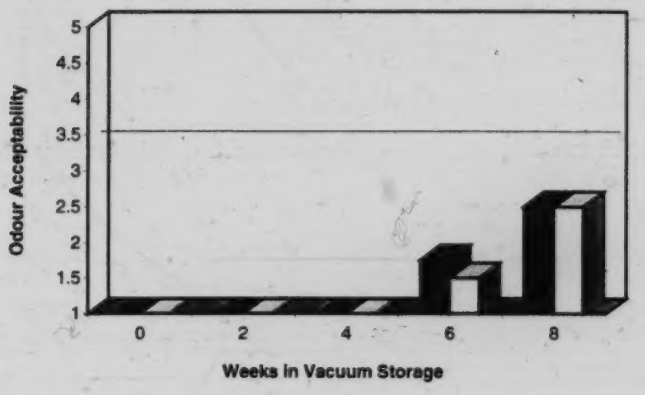
Storage life was assessed by odour and appearance. VP pork loins and butts did not reach a point of rejection based on evaluation of appearance during vacuum storage at -1°C or 2°C. Figure 3.3 shows the odour acceptability of pork loins after vacuum storage at either -1 or 2°C. The data clearly show that there were very few appreciable differences in odour between CT and NT loins during the vacuum storage at -1 or 2°C. Acceptability scores for CT loins and NT loins did not reach 3.5 on the odour acceptability scale after 8 weeks under vacuum at -1°C. Consequently, CT loins and NT loins have a storage life based on odour of > 8 weeks. At 2°C acceptability scores for CT and NT loins reached 3.5 on the odour acceptability scale after 6 weeks of vacuum storage; therefore both CT and NT pork loins have a storage life based on odour, of 6 weeks at 2°C.

Figure 3.4 shows the acceptability of odour of VP pork butts after storage at two temperatures. The data clearly show that there were very few appreciable differences in odour between CT and NT butts during storage at -1 or 2°C. Acceptability scores for CT and NT pork butts reached 3.5 on the odour acceptability scale after 8 weeks of storage at -1°C and after 6 weeks of storage at 2°C. Consequently the storage life based on odour, for pork butts stored at -1°C and 2°C was 8 weeks and 6 weeks, respectively.

3.3.3. Bacterial growth during simulated aerobic retail display

Results for treatment effects were similar for 0, 2, 4, 6, and 8 weeks of vacuum storage at -1°C and for 0, 2, 4, and 6 weeks at 2°C. For the purpose of demonstration, 4 weeks was selected for presentation and discussion. The data for the bacteriology and pH

A) –1°C



B) 2°C

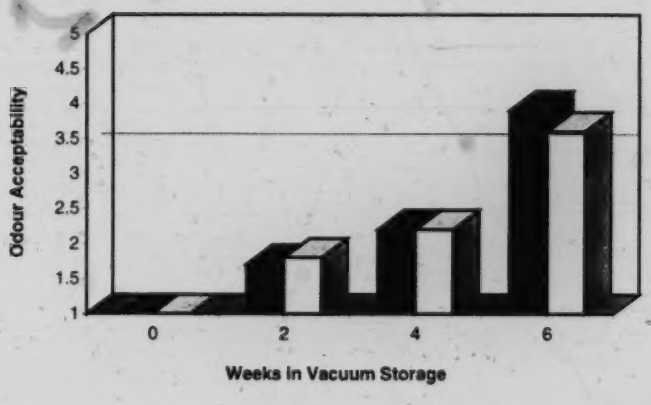
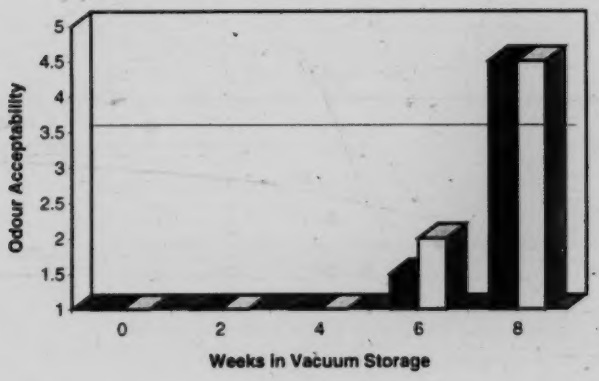


Figure 3.3. Odour acceptability of VP pork loins after storage at A) -1°C and B) 2°C. Loins are considered to be spoiled when scores reach 3.5 on the odour acceptability scale. Five = unacceptable; three = neither acceptable or unacceptable; one = acceptable. Black bars = VP pork loins that were conveyed through the CO_2 chill tunnel (CT). White bars = VP pork loins that were not conveyed through the CO_2 chill tunnel (NT). Each bar represents the mean of 20 scores (5 panelists, two samples, and two replications).

A) -1°C



B) 2°C

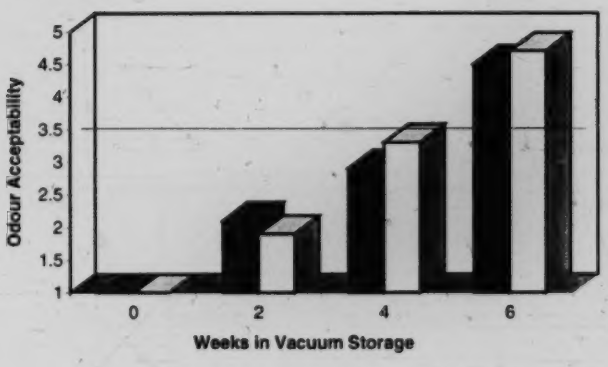


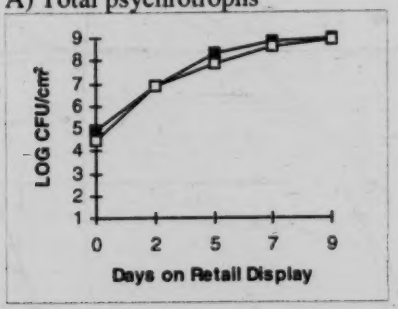
Figure 3.4. Odour acceptability of VP pork butts after storage at A) -1°C and B) 2°C. Butts are considered to be spoiled when scores reach 3.5 on the odour acceptability scale. Five = unacceptable; three = neither acceptable or unacceptable; one = acceptable. Black bars = VP pork butts that were conveyed through the CO_2 chill tunnel (CT). White bars = VP pork butts that were not conveyed through the CO_2 chill tunnel (NT). Each bar represents the mean of 20 scores (5 panelists, two samples, and two replications).

of the retail cuts at all vacuum storage times are tabulated in Appendix 2 to 5. Figure 3.5 shows the growth of bacteria during retail display at 8°C of pork loin slices prepared from pork loins previously stored for 4 weeks under vacuum at -1°C. Increased time in the retail case resulted in an increase in the numbers of all bacterial groups enumerated and growth was initiated without an apparent lag phase for all of the bacterial groups. Growth of LAB was essentially the same as that of the total psychrotrophic bacterial population. Both of these groups increased from about log 5.0 CFU/cm^2 to near log 9.0 CFU/cm^2 during the 9-day retail display period. *Pseudomonas* spp. emerged as the next most dominant bacterial population during the 9 day retail display period growing from about log 2.0 CFU/cm^2 to log 8.0 CFU/cm^2. *Enterobacteriaceae* and *Brochothrix thermosphacta* grew from populations of about log 1.0 CFU/cm^2 to log 6.0 CFU/cm^2 during the retail display period.

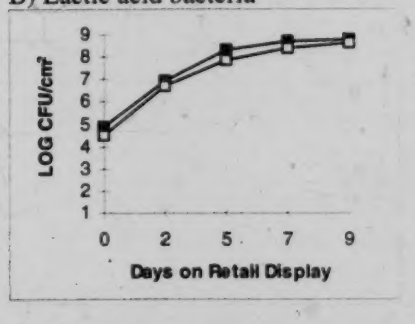
The two chilling treatments (CT and NT) did not affect the bacterial growth for any of the five bacterial groups enumerated. There were a few inconsistent differences in bacterial numbers but all of these were less than one log count difference, with the exception of one instance where *Pseudomonas* spp. were almost 2 log units higher for the CT loin slices. However, these differences could not be attributed to loin chilling treatment. These results were not surprising because there was no difference in the initial bacterial numbers on the surface of loin slices at the onset of the retail display period and the main factor influencing bacterial growth during retail display was retail case temperature, not subprimal chilling treatment.

Figure 3.6 compares the growth of bacteria during retail display of pork loin slices prepared from VP pork loins previously stored for 4 weeks at 2°C. Increased time in the retail case resulted in an increase in the numbers of all bacterial groups enumerated and this was initiated without an apparent lag phase for all bacterial groups with exception of *Pseudomonas* spp. which exhibited at least a 48 hour lag phase. Growth of LAB closely paralleled the growth of total psychrotrophic bacteria. Both of these groups increased

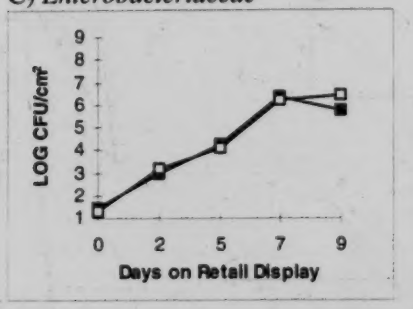
A) Total psychrotrophs



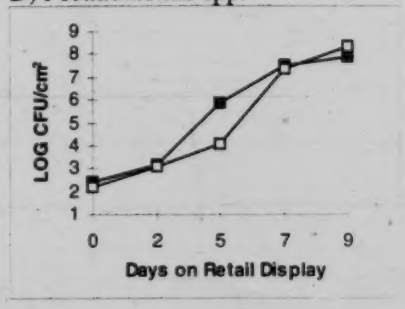
B) Lactic acid bacteria



C) *Enterobacteriaceae*



D) *Pseudomonas* spp.



E) *Brochothrix thermosphacta*

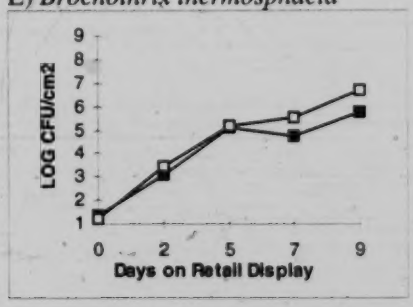
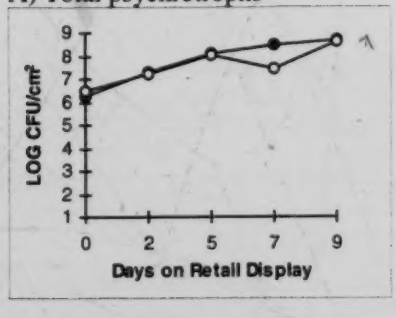
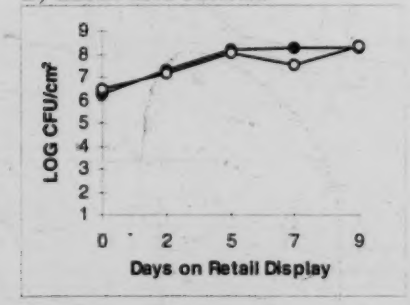


Figure 3.5. Effect of time on aerobic retail display at 8°C on the growth of A) total psychrotrophs; B) lactic acid bacteria; C) *Enterobacteriaceae*; D) *Pseudomonas* spp.; E) *Brochothrix thermosphacta* on pork loin slices prepared from loins that were stored under vacuum for 4 weeks at -1°C. Solid symbols = slices obtained from loins conveyed through the CO_2 chill tunnel (CT). Open symbols = slices obtained from pork loins that were not conveyed through the CO_2 chill tunnel (NT). Each data point represents the mean of 4 samples (2 samples for each of 2 replications).

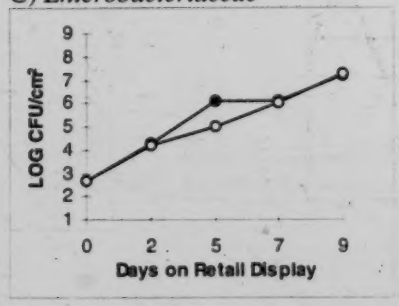
A) Total psychrotrophs



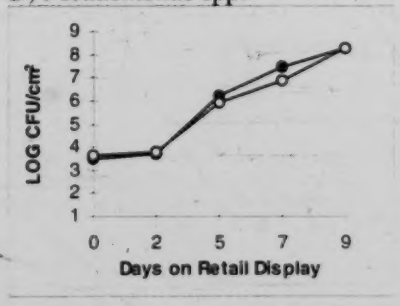
B) Lactic acid bacteria



C) *Enterobacteriaceae*



D) *Pseudomonas* spp.



E) *Brochothrix thermosphacta*

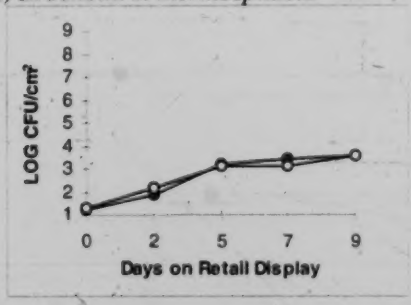


Figure 3.6. Effect of time on aerobic retail display at 8°C on the growth of A) total psychrotrophs; B) lactic acid bacteria; C) *Enterobacteriaceae*; D) *Pseudomonas* spp.; E) *Brochothrix thermosphacta* on pork loin slices prepared from loins that were stored under vacuum for 4 weeks at 2°C. Solid symbols = slices prepared from pork loins conveyed through the CO_2 chill tunnel (CT). Open symbols = slices prepared from pork loins that were not conveyed through the CO_2 chill tunnel (NT). Each data point represents the mean of 4 samples (2 samples for each of 2 replications).

from about log 6.0 CFU/cm^2 to over log 8.0 CFU/cm^2 during the 9 day retail display period. After the 48 hour lag phase *Pseudomonas* spp. grew from an initial population of about log 4.0 CFU/cm^2 to over log 8 CFU/cm^2, joining LAB as the dominant bacterial population. *Enterobacteriaceae* emerged as the next dominant population growing from 3.0 CFU/cm^2 to about log 7.0 CFU/cm^2 during retail display. *B. thermosphacta* grew slowly over the 9 day retail display period from log 1.0 CFU/cm^2 to about log 3.5 CFU/cm^2.

Chilling treatment did not have an effect on the bacterial growth of any of the 5 populations enumerated. As noted with loin slices prepared from loins previously stored at -1°C, differences were inconsistent and could not be attributed to chilling treatment. Again, this is not surprising because CT and NT initial populations were almost identical for the 5 bacterial populations enumerated.

Figure 3.7 compares the growth of bacteria during retail display of pork butt slices prepared from pork butts previously stored for 4 weeks in vacuum at -1°C. Increased time on retail display resulted in an increase in the numbers of all bacteria enumerated and occurred without an apparent lag phase. As with loin slices the growth of LAB was essentially the same as that of the total psychrotrophs. These two groups increased from about log 6.0 CFU/cm^2 6 to log 9.0 CFU/cm^2 during retail display. *Pseudomonas* spp. became the other dominant population increasing from log 3.0 CFU/cm^2 to about log 9.0 CFU/cm^2 during the display period. *Enterobacteriaceae* increased from log 3.0 CFU/cm^2 to log 7.0 CFU/cm^2 after 7 days on retail display while *B. thermosphacta* increased from log 3.0 CFU/cm^2 to log 7 CFU/cm^2 after 5 days on retail display.

CT butt slices showed higher numbers during retail display which can most likely be attributed to the fact that CT butts stored for 4 weeks under vacuum at -1°C (see Figure 3.2) had high populations of all groups of bacteria enumerated. However, these differences could not be attributed to chilling treatment of the butts because it is very unlikely that the colder temperature regime experienced by CT butts would increase

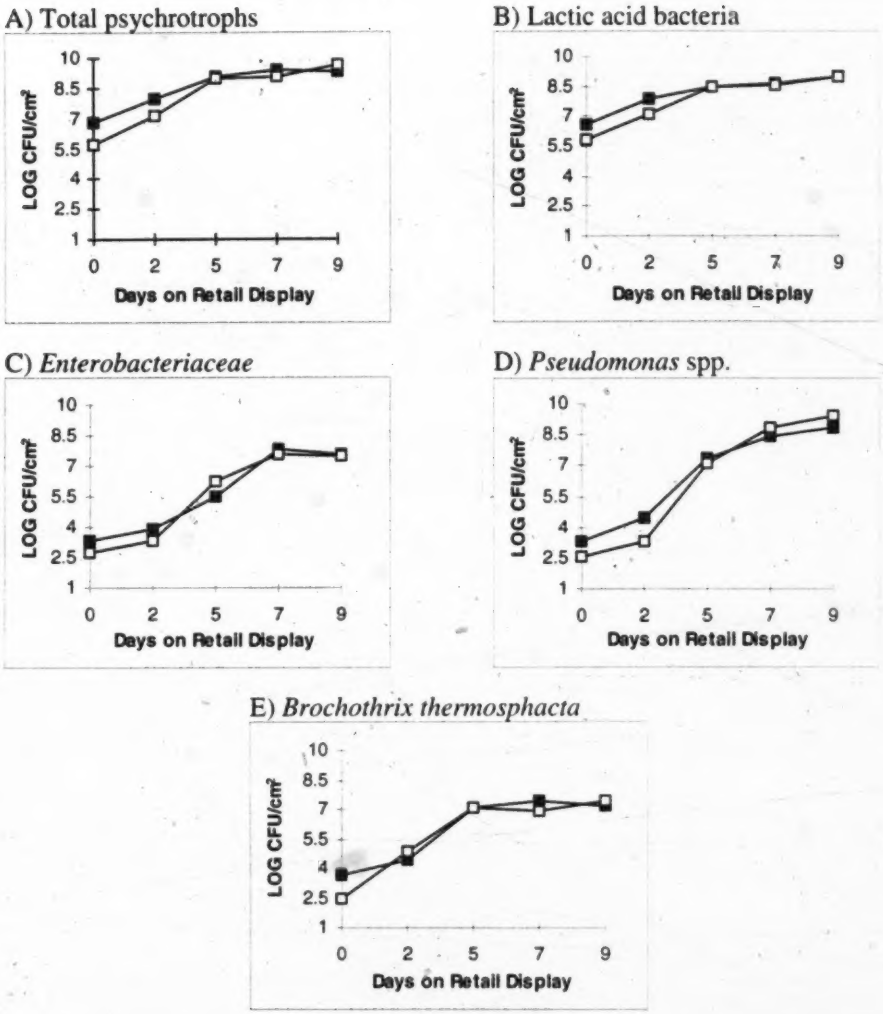


Figure 3.7. Effect of time on aerobic retail display at 8°C on the growth of A) total psychrotrophs; B) lactic acid bacteria; C) *Enterobacteriaceae*; D) *Pseudomonas* spp.; E) *Brochothrix thermosphacta* on pork butt slices prepared from butts that were stored under vacuum for 4 weeks at -1°C. Solid symbols = slices prepared from pork butts that were conveyed through the CO_2 chill tunnel (CT). Open symbols = slices prepared from butts that were not conveyed through the CO_2 chill tunnel (NT). Each data point represents the mean of 4 samples (2 samples for each of 2 replications).

bacterial growth and that the higher counts on CT butts occurred only at the one vacuum storage time.

Figure 3.8 compares the growth of bacteria during retail display of pork butt slices prepared from pork butts previously stored for 4 weeks in vacuum at 2°C. Increased time on retail display resulted in an increase in the numbers of all bacterial groups enumerated and was initiated without an apparent lag phase for the 5 bacterial groups. Growth of LAB was very close to that of the total psychrotroph bacterial population. Both of these groups increased from about log 7.0 CFU/cm^2 to near log 9.0 CFU/cm^2 during retail display. *Pseudomonas* spp. also grew to log 9.0 CFU/cm^2 but was initially at log 4.0 CFU/cm^2. *Enterobacteriaceae* numbers were also initially at log 4.0 CFU/cm^2 but only grew to about log 7.0 CFU/cm^2. The two chilling treatments did not affect the bacterial growth for any of the five bacterial groups enumerated.

3.3.4. Retail case life

Figure 3.9A shows the effect of storage time under vacuum on the retail case life of pork loin slices based on odour. Generally, for both treatments, the retail case life in days decreased as time in vacuum storage increased. Retail case life was similar for slices prepared from vacuum packaged CT and NT loins at each storage interval except at 2 weeks where the slices prepared from NT vacuum packaged loins had a retail case life that was 1.6 days lower than CT slices. Retail case life of slices prepared from CT and NT loins stored in vacuum at 2°C (Figure 3.9B) was similar at each storage interval and declined as vacuum storage time increased.

Figure 3.10 shows the effect of storage under vacuum A) -1°C and B) 2°C on the retail case life of slices prepared from pork butts. Both graphs clearly show the similarity in retail case life of CT and NT slices, as well as a negative correlation between time in vacuum storage and retail case life. After six weeks of vacuum storage at -1°C slices prepared from CT vacuum packaged pork butts had a retail case life of 0.6 days greater

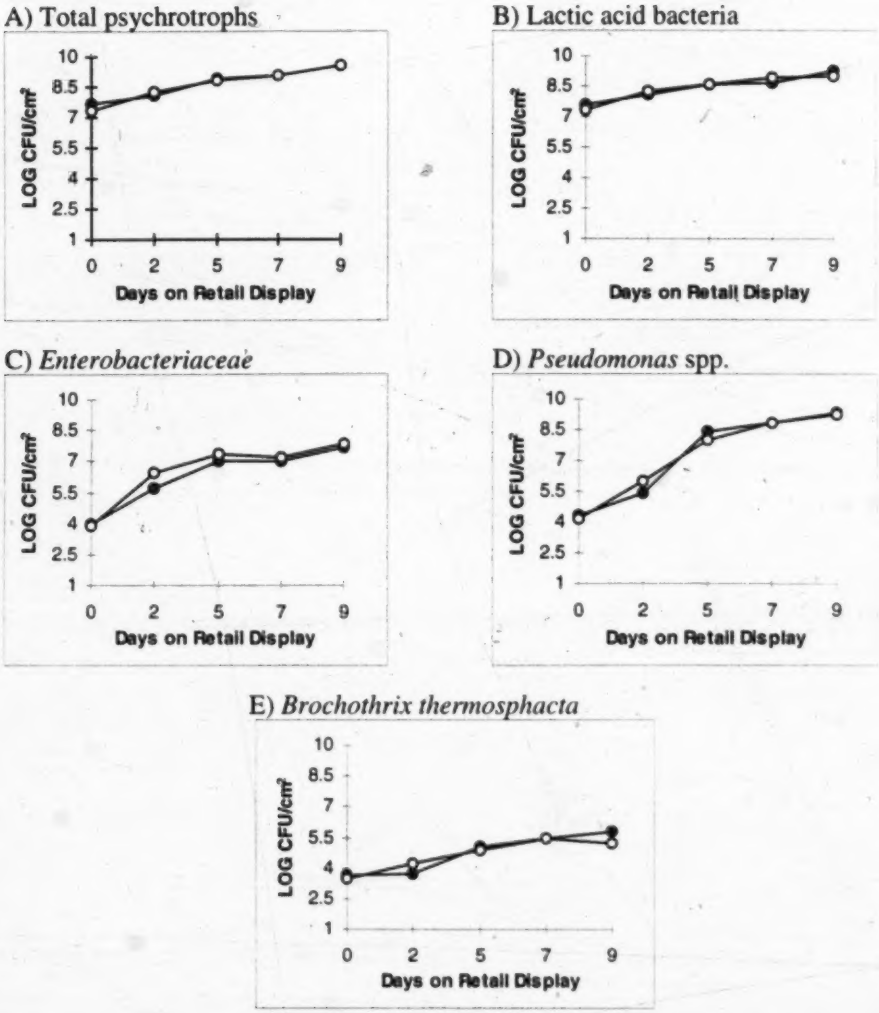
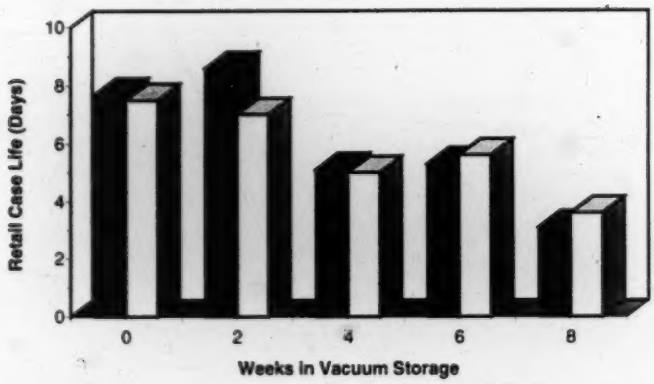


Figure 3.8. Effect of time on aerobic retail display at 8°C on the growth of A) total psychrotrophs; B) lactic acid bacteria; C) *Enterobacteriaceae*; D) *Pseudomonas* spp.; E) *Brochothrix thermosphacta* on pork butt slices prepared from butts that were stored under vacuum for 4 weeks at 2°C. Solid symbols = slices prepared from butts that were conveyed through the CO_2 chill tunnel (CT). Open symbols = slices prepared from butts that were not conveyed through the CO_2 chill tunnel (NT). Each data point represents the mean of 4 samples (2 samples for each of 2 replications).

A) -1°C



B) 2°C

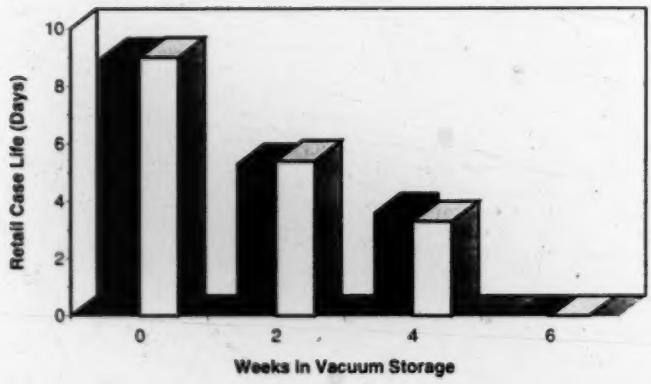
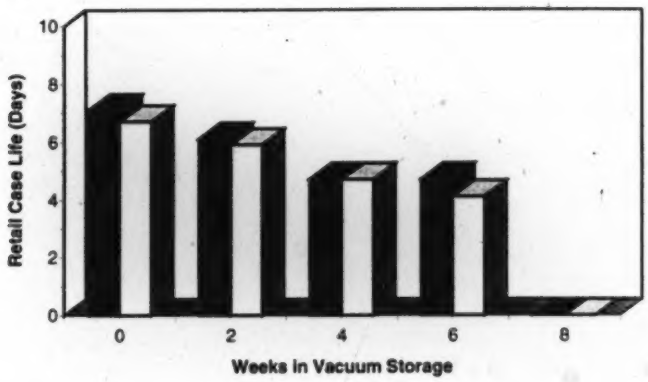


Figure 3.9. Retail case life based on odour of pork loin slices prepared from loins that were stored under vacuum for up to 8 weeks at A) -1°C and B) 2°C and stored aerobically in a retail case at 8°C. Black bars = slices prepared from loins that were conveyed through the CO_2 chill tunnel (CT). White bars = slices prepared from loins that were not conveyed through the CO_2 chill tunnel (NT). Case life was based on the evaluation of 10 samples (5 panelists and 2 replications).

A) -1°C



B) 2°C

Figure 3.10. Retail case life based on odour of pork butt slices prepared from butts that were stored under vacuum for up to 8 weeks at A) -1°C and B) 2°C and stored aerobically in a retail case at 8°C. Black bars = slices prepared from butts that were conveyed through the CO_2 chill tunnel (CT). White bars = slices prepared from butts that were not conveyed through the CO_2 chill tunnel (NT). Case life was based on the evaluation of 10 samples (5 panelists and 2 replications).

than slices from NT vacuum packaged butts, and after 2 weeks in vacuum storage at 2°C when CT loins showed a retail case life of 0.8 days less than NT butt slices.

3.3.5. Sensory evaluation

The overall flavour intensity of the pork loins stored at either -1 or 2°C did not significantly change over the 8 or 6 weeks of storage (Figure 3.11A,B). The flavour intensity of the pork butts stored at -1°C did not change over the 8 weeks of storage (Figure 3.11C); however, pork butts stored at 2°C had a stronger ($p<0.05$) overall flavour intensity than the coded reference sample after 4 weeks of storage (Figure 3.11D). The chill tunnel had no effect on the overall flavour intensity scores of the pork loins (Figure 3.11). The intensity of the off-flavour notes (Appendices 6 to 9) did not change with storage time and there were no significant differences between treatments.

3.3.6. Purge accumulation

The data in Table 3.1 compares the accumulation of purge in VP pork loins stored at -1 and 2°C. Initially (0 weeks) under a vacuum at -1°C, NT loins showed significantly ($p<0.05$) higher purge levels than CT counterparts. Purge level (% by weight) was 0.36 for CT loins and 0.56 for NT loins. After 6 weeks in vacuum storage at 2°C, CT loins showed significantly ($p<0.05$) higher purge levels than NT counterparts. CT purge level was 7.20 % by weight compared with 5.65% for NT loins. No other statistical differences were observed.

The data in Table 3.2 compare the accumulation of purge in VP pork butts stored at -1 and 2°C. After 4 weeks of storage at -1°C, CT butts showed a purge level of 2.02 % by weight, which was significantly higher ($p<0.05$) than NT counterparts (0.70 % by weight). No other statistical differences were observed. Statistical comparisons were not made, but pork butts definitely had lower purge levels than pork loins.

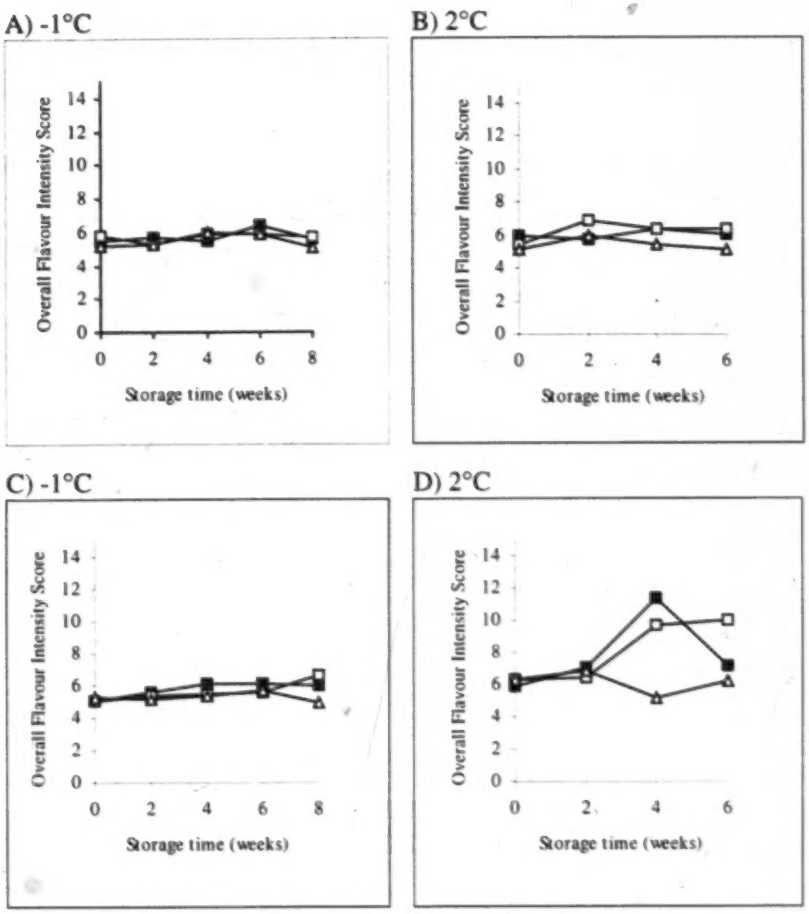


Figure 3.11. Mean flavour intensity scores (0=none, 15=very strong) after storage at -1°C (A, C) and 2°C (B, D) of a freshly prepared reference sample (Δ) and of VP pork loins (A, B) and butts (C, D) that were conveyed through the CO_2 chill tunnel (■; CT) or not conveyed through the chill tunnel (□; NT). Means are averages of forty determinations (2 replicates x 2 samples x 10 panelists).

Table 3.1. Effect of the CO_2 chill tunnel on the accumulation of purge in vacuum packaged pork loins stored at –1 and 2°C.

Weeks in Vacuum Storage	Total purge (% by weight)			
	-1°C		2°C	
	CT[1]	NT[2]	CT	NT
0	0.36[B]	0.56[A]	2.35	1.40
2	3.69	2.47	5.86	4.37
4	3.16	3.31	7.92	5.96
6	3.94	4.01	7.20[A]	5.65[B]
8	4.15	5.97	-	-

[AB] Means within the same row, for the same storage temperature, with different letters are significantly different ($p<0.05$).
Means are averages of 4 determinations (2 for each of 2 replications).
[1] Primal cuts were exposed to the CO_2 chill tunnel (CT).
[2] Primal cuts were not exposed to the CO_2 chill tunnel (NT).
- Not tested.

Table 3.2. Effect of the CO_2 chill tunnel on the accumulation of purge in vacuum packaged pork butts stored at –1 and 2°C.

Weeks in Vacuum Storage	Total purge (% by weight)			
	-1°C		2°C	
	CT[1]	NT[2]	CT	NT
0	0.40	0.32	0.52	0.49
2	1.39	1.07	0.99	0.77
4	2.02[A]	0.70[B]	1.92	1.75
6	1.81	1.44	2.75	2.79
8	2.16	2.59	-	-

[AB] Means within the same row with different letters are significantly different ($p<0.05$). Means are averages of 4 determinations (2 for each of 2 replications).

[1] Primal cuts were exposed to the CO_2 chill tunnel (CT).

[2] Primal cuts were not exposed to the CO_2 chill tunnel (NT).

- Not tested.

3.4. DISCUSSION

Weakley et al. (1986) reported that rapid chilling of VP pork loins, resulting in a 'crust freeze', did not have a beneficial effect on the microbiology during storage at 2°C. The present study supported these results because VP pork loins and butts that were conveyed through a CO_2 chill tunnel, resulting in a crust freeze, did not show improved microbial quality during storage compared with counterparts that were not conveyed through the CO_2 chill tunnel prior to storage. Chapter 2 reported that fresh VP pork loins that were conveyed through the CO_2 chill tunnel prior to being placed in boxes had a dramatically lower surface temperature compared with VP pork loins that were placed in boxes after exiting the heat shrink, without exposure to the CO_2 chill tunnel. In light of this finding, and the fact that bacterial metabolism and, therefore, growth are highly temperature dependent (Zamora and Zaritzky, 1985) it would be expected that bacterial proliferation on NT pork loins and butts would be greater than on CT counterparts during vacuum storage. However, the results of the present study do not support this. These results can be explained by the fact that after *ca.* 24 hours of vacuum storage the surface temperature of the CT and NT primal cuts had converged to very close to the same temperature. This similarity of surface temperature for CT and NT product after *ca.* 24 hours of storage can be attributed to minimal insulation effect, because only 9 boxes of meat were stored, thus allowing heat from the NT product to rapidly disperse into the surrounding air. Consequently, the difference in surface temperature between CT and NT pork loins and butts in the present study does not persist long enough to affect bacterial growth. Judging from the number of pork loins used by Weakley et al. (1986) the insulation effect would be similar to that observed in the present study. Chapter 5 of this thesis reports the effects of a substantial insulation effect during commercial shipping conditions where a large meat mass was investigated.

From a practical perspective, the significant differences in bacterial growth between CT and NT loins and butts during vacuum storage would not be meaningful. Differences were often less than 1 log CFU/cm^2 and had a negligible effect on product quality. Furthermore, the differences were random over storage temperatures and times. Differences in bacterial counts, whether statistically significant or visually noticeable from the growth curves can be attributed to factors other than the effect of the CO_2 chill tunnel. Such as pH, initial contamination load of a particular primal cut, and limitations of bacterial sampling.

The prevalence of LAB during the anaerobic storage of chilled meats is well established (Hitchener et al., 1982; Shaw and Harding, 1984; Schillinger and Lücke, 1987a,b; Borch and Molin, 1988; Dainty and Mackey, 1992). In the current investigation, LAB were the dominant organisms during vacuum storage at -1°C and 2°C. Egan and Shay (1984) and Egan et al. (1986) reported that gram-negative bacteria comprise a greater proportion of the flora for VP pork stored at 5°C compared with 0°C. These results were supported by the present study because presumptive *Enterobacteriaceae* and *Pseudomonas* spp. reached higher populations in vacuum storage at 2°C than at -1°C.

Unlike storage life based on appearance, which often depends on nonbacterial factors, storage life based on odour acceptability is primarily a function of bacterial metabolism and growth. Thus, it is not surprising that for both primal cuts and both storage temperatures the storage life of CT and NT vacuum packaged pork, based on odour, was the same. For both pork loins and butts and for both treatments the storage life decreased when stored at the warmer of the two storage temperatures. This was also reported by Egan et al. (1986) because VP pork loins stored at 5°C had a storage life that was 2 to 3 weeks shorter than at 0°C. The present study showed that for both treatments pork butts have a shorter storage life than pork loins under vacuum at both storage temperatures. This can be attributed to the fact that pork butts had a higher pH (pH>6.0)

resulting in a more favourable environment for bacterial growth; particularly *Enterobacteriaceae* and *B. thermosphacta*, both of which can contribute to early spoilage of VP high pH (pH>6), glucose deficient dark, firm, dry (DFD) meat, resulting from the production of offensive off-odours.

In this study, especially with increased storage time under vacuum, LAB remained one of the two dominant bacterial populations during the 9 day retail display period. LAB were reported to be the dominant organisms on pork chops prepared from loins stored anaerobically for up to 24 weeks (Greer et al., 1993). The current study found that presumptive *Pseudomonas* spp. grew rapidly during aerobic retail display and emerged as the second most important bacterial population. Similar results were also reported by Greer et al. (1993); however, in that study, *Pseudomonas* spp. made up a smaller proportion of the total bacterial population during retail display than in the current study. Greer et al. (1993) utilized a strict anaerobic environment with a high CO_2 concentration for the storage of pork loins prior to preparation for retail display, probably resulting in no growth of *Pseudomonas* spp. during prolonged anaerobic storage. In the current study, presumptive *Pseudomonas* spp. showed some level of growth during vacuum storage in all instances except for pork loins stored under vacuum at -1°C.

During preparation of pork slices for retail display care was taken to ensure that bacterial contamination was only derived from the primal cuts themselves; thus, the bacterial load of the slices should be a function of the bacterial load on the primal cut. It would be expected that bacterial growth during aerobic retail display for both treatments would be very similar, because the bacterial load on CT and NT primal cuts at the time of slicing was similar. Although the CO_2 chill tunnel appeared to have no effect on bacterial growth during vacuum storage and contamination of slices was derived from the primal cuts, differences in growth of bacteria on CT and NT pork slices could be due to intrinsic factors, particularly pH. For example, the higher bacterial populations reported for NT loin slices prepared from loins stored for 2 weeks at 2°C can be attributed to the fact that

the slices prepared for retail display came from loins with a uncharacteristically high pH (pH>6.1). This results in loin slices with a pH much higher than the CT slices, which had lower bacterial counts. The same explanation cannot be used to explain the higher bacterial counts on NT loin slices prepared from loins stored under vacuum at -1°C for 2 weeks, because the pH of the CT and NT slices were similar. However, due to the fact that CT and NT loins stored under vacuum at this interval had similar microbial loads it can be assumed that the NT slices were somehow contaminated during preparation for aerobic retail display, resulting in the higher counts reported during aerobic retail display. This may explain the decreased retail case life of the NT loin slices prepared from NT loins stored at -1°C for 2 weeks. The greater initial bacterial load on these slices caused more rapid deterioration in odour acceptability because of production of odorous compounds by the high numbers of bacteria. The higher numbers of bacteria reported on loin slices prepared from NT loins stored for 2 weeks at 2°C did not cause the decreased aerobic retail case life because samples for replicate 2 NT loin slices at the two week storage interval was slightly longer than for CT slices; thus the mean retail case life for both replications was very close to the same for CT and NT slices (Figure 3.9B). The retail case life of CT and NT pork loin and butt slices was similar in all other situations. This would be expected because acceptability in terms of odour is largely a function of bacterial growth. The current study supports the findings of Greer at al. (1993) who showed that prolonged anaerobic storage compromises the retail case life of pork packaged aerobically after removal from anaerobic storage.

Because there were very few significant differences ($p<0.05$) between the flavour of CT and NT pork loins and butts in this study, it can be concluded that the CO_2 chill tunnel had no effect on the flavour attributes of VP pork loins and butts. These findings support the results of Wiley et al. (1989) in which rapid chilling of hot boned pork loins had no effect on sensory traits. Furthermore, the results of the present study support the

findings of Weakley et al. (1986) in which rapid chilling, resulting in a crust freeze of VP pork loins had no beneficial effect on palatability of chops.

It is commonly thought that freezing of muscle tissue increases purge accumulation in meats (Penny, 1974; Añón and Calvelo, 1978; Jalong'O et al., 1987; Greer and Murray, 1991). Weakley et al. (1986) showed that rapid chilling with propylene glycol, resulting in a crust freeze, increased purge in hot processed VP pork loins while crust freezing in a blast freezer did not. Wiley et al. (1989) reported that hot processed pork loins and butts that were chilled in a CO_2 chill tunnel, resulting in a crust freeze, showed higher purge than their conventionally (cold) processed counterparts. The present study showed that the CO_2 chill tunnel had little effect on purge in VP pork loins and butts. In a number of situations the CT cuts appeared to have higher levels of purge, but these differences were not significantly different ($p>0.05$). This may have resulted from the fact that there was extremely high variability in the purge levels among individual samples, even with the same treatment. For example, the purge in one CT loin sample after 4 weeks in vacuum storage was almost 10% higher than the second CT sample for the same vacuum storage interval, resulting in an extremely high standard error of the mean. Consequently, due to the high variability among pork loin and butt samples conclusions could not be drawn with respect to the effect of the CO_2 chill tunnel on purge accumulation in VP pork loins and butts obtained from normal daily product at a large pork abattoir. Chapter 4 of this thesis assesses the effect of the CO_2 chill tunnel on the accumulation of purge in VP pork loins that were of controlled muscle quality in an attempt to limit variability among samples.

The results of this study indicate that rapid chilling of conventionally processed VP pork loins and butts did not have a beneficial or a detrimental effect on bacteriology of the meat during vacuum storage, or on the storage life based on odour. Also that the bacteriology during aerobic retail display, retail case life, flavour attributes, and accumulation of purge were not affected by chilling treatment.

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4. Effect of a CO_2 Chill Tunnel on the Accumulation of Purge in Vacuum Packaged Pork Loins

4.1. INTRODUCTION

From a storage life perspective vacuum packaging (VP) of fresh, chilled meats has revolutionized the fresh meat industry. Vacuum packaging combined with low temperature storage has the potential to extend the storage life of fresh meat for many weeks longer than is possible with aerobic packaging. As a result, many North American, including a number of Alberta pork producers, have capitalized on profitable export markets where shipping time can be extensive. Unfortunately, VP provides an environment in which meat is considered to be susceptible to accumulation of a reddish liquid in the vacuum package; this liquid is known as exudate, drip, or purge. Purge is considered to be unattractive and is often associated with low quality fresh meats. Japanese importers of North American pork have expressed concern over large amounts of purge accumulation in the packages of VP pork shipped from an Alberta pork abattoir. Because of the profitability of the Japanese export market, Alberta pork producers are interested in the factors in pork processing that contribute to increased levels of purge in VP pork.

Packaged pork exiting the CO_2 chill tunnel is 'crust frozen' meaning that the first few millimeters of the tissue surface are frozen. It is commonly thought that freezing of muscle tissue accounts for increased purge (Penny, 1974; Jalong'O et al., 1987; Greer and Murray, 1991); however, little information exists in the scientific literature on the effect of 'crust freezing' on the accumulation of purge in VP pork. Weakley et al. (1986) reported that 'crust freezing' of hot boned pork loins by submersion in -20°C propylene glycol for 1 hour increased purge, while 'crust freezing' of hot boned pork loins in a blast freezer at -30°C for 1 hour did not increase purge. Wiley et al. (1989) reported that a

crust freeze of hot boned pork loins and shoulders in a CO_2 chill tunnel (-79°C) resulted in higher levels of purge compared with normally chilled, conventionally processed (2°C chilling for 24 hours after cold-boning) counterparts. Both of these studies involved chilling periods of 15 minutes or greater. Little information exists on the effect of rapid chilling (<5 minutes) on purge accumulation in VP pork.

The objective of this study was to determine the effect of a CO_2 chill tunnel utilized by an Alberta pork abattoir on the accumulation of purge in VP pork loins of similar pH. Product of known quality was used and a design adopted to reduce variability described in Chapter 3 was used.

4.2. MATERIALS AND METHODS

Five boneless pork loins of normal pH (5.5 to 5.8) were obtained *ca.*12 hours after hogs were slaughtered using conventional methods at the federally inspected, research abattoir of Agriculture and Agri-Food Canada's Lacombe Research Centre. Immediately after removal of the loins, they were wrapped with oxygen permeable plastic wrap and stored for 12 h in a cooler with an air temperature of 2°C. Following cold storage one of the loins was removed from the cooler and cut into 6 sections. Each section was patted dry with tissue paper, weighed and VP in high barrier PVDC/polyethylene vinyl alcohol bags using a Multivac (Sepp Hagenmuller KG, Wolfert Schwender, Germany) VP machine.

The first, third, and fifth sections that were weighed and VP were labeled CT for chilling in the CO_2 chill tunnel, while the second, fourth, and sixth sections were labeled NT indicating that they were not chilled in the CO_2 chill tunnel. The same weighing, packaging and labeling procedures were also done for the four remaining loins. Each of the sections from the first loin were labeled with '0' for zero weeks in vacuum storage and the sections from the remaining four loins were randomly labeled with '2', '4', '6', and '8' weeks.

After the labeling was complete the 15 CT sections and the 15 NT sections were placed in separate coolers and transported, on ice, to the commercial abattoir in Red Deer, Alberta and the 15 CT loin sections were passed through the CO_2 chill tunnel in approximately 3 minutes (0.08 m/sec). Upon exiting the chill tunnel they were placed on ice in the cooler. Both coolers were returned to the Lacombe Research Station where the 15 CT sections and the 15 NT sections were placed in separate boxes. Both boxes were stored for 8 weeks at -1°C. After storage intervals of 0, 2, 4, 6 and 8 weeks the appropriately labeled loin sections were removed from storage to measure purge accumulation.

Sections were removed from vacuum, patted dry, and weighed as previously described. The difference between the original dry weight and the dry weight after the storage interval represents the weight of purge loss, which was expressed as percent purge by weight. All comparisons between CT and NT were made on adjacent sections from a single loin. The experiment was replicated twice for vacuum storage at -1°C and three times for storage at 2°C. Loin sections were stored for 0, 2, 4 and 6 weeks for the 2°C study. The mean pH of CT loin sections measured during the initial weighing was 5.56 and the mean pH of the NT loin sections was 5.55.

4.3. RESULTS

Table 4.1. shows the effect of the CO_2 chill tunnel on the accumulation of purge for VP pork loin sections stored at two temperatures. Purge loss was not statistically different ($P>0.05$) between the two chill treatments during vacuum storage except for the initial week at -1°C. Purge losses increased with storage temperature and for both treatments from 0 to 2 weeks in vacuum storage. From 2 weeks of vacuum storage until the end of the storage period, purge levels remained between 3.0% and 4.2% for both storage temperatures and for both treatments.

Table 4.1. Effect of the CO_2 chill tunnel on purge loss in vacuum packaged sections of pork loin.

Storage time in vacuum (weeks)	PURGE LOSS (% by weight)			
	-1°C Vacuum Storage[1]		2°C Vacuum Storage[2]	
	Chill Tunnel (CT)	No Chill Tunnel (NT)	Chill Tunnel (CT)	No Chill Tunnel (NT)
0	1.1[B]	1.5[A]	1.3	1.4
2	3.3	3.2	3.7	3.8
4	4.2	3.9	3.3	3.7
6	3.0	3.1	3.5	3.5
8	3.8	4.0	-	-

[A,B] Means in the same row with different superscripts are different ($P<0.05$).
[1] Data are the least squares means of 6 loin sections.
[2] Data are the least squares means of 9 loin sections.
- Not tested.

4.4. DISCUSSION

The fact that freezing of muscle tissue contributes to increased purge in VP meats is well documented (Penny, 1974; Jalong'O et al., 1987; Greer and Murray, 1991). However, this study showed that 'crust freezing' of fresh VP pork loin sections after passing through a CO_2 chill tunnel which operates at approximately -80°C, did not cause significant increases in purge losses from the meat. In fact, in a number of instances loin sections that were not passed through the CO_2 chill tunnel had a trend to higher loss of purge than their counterparts that were passed through the CO_2 chill tunnel.

These unexpected findings can possibly be explained by the freezing rate of the surface of the loin sections. When muscle tissue is frozen quickly, as occurs with exposure to the CO_2 chill tunnel, ice crystals form intracellularly (Anon and Calvelo, 1980). Alternatively, when muscle tissue freezes over longer periods of time, crystallization is largely extracellular (Anon and Calvelo, 1980). Consequently, water is drawn out of cells to form the extracellular ice which causes dehydration and distortion of cells. It is this distortion that results in decreased water reabsorption capacity of cells upon thawing, ultimately leading to increased levels of purge.

It appears that 'crust freezing' of pork loin sections by passing through a CO_2 chill tunnel results in rapid freezing and the formation of intracellular ice crystals. Consequently, the CO_2 chill tunnel does not appear to cause increased purge in VP pork loins.

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5. Effect of a CO_2 Chill Tunnel on the Temperature Regime of Boxed Pork Loins During Simulated Commercial Transport

5.1. INTRODUCTION

Vacuum packaging extends the storage life of fresh pork for weeks past that which is possible with traditional, aerobic packaging. This extension of storage life has resulted in an explosion in the number of abattoirs that utilize VP technology. Vacuum packaging is particularly important for local pork processors who ship fresh, unfrozen pork to distant, overseas markets, such as Japan. A number of Alberta pork producers have adopted a technologically advanced packaging process that was characterized in Chapter 2. Prior to the work reported in this thesis, little information existed in the scientific literature on the effects of commercial packaging on the microbiology and storage life of VP pork. Data reported in Chapter 3 showed that rapid chilling of fresh VP pork loins and butts by passing the packaged product through a CO_2 chill tunnel had no effect on the bacteriology or storage life of these products. Similar results were reported by Weakley et al. (1986) for rapidly chilled, hot boned pork loins with a crust freeze. Rapid chilling did not show improved shelf life compared with normally chilled counterparts (Weakley et al., 1986). The results of this study and the data presented in Chapter 3 appear to discount the need for rapid chilling of fresh pork with CO_2 prior to boxing and shipment. However, these studies were done under controlled conditions with limited quantities of boxed meat (approximately 10 boxes) and these conditions did not reflect commercial practice. In a commercial situation when large numbers of boxes (100's) are stored or transported in close proximity, as is the case with a refrigerated trailer going to Japan. It is conceivable that there will be a substantial insulation effect, especially for boxes positioned in the centre of the refrigerated trailer. Prior to concluding that the CO_2 chill tunnel had no beneficial effect on the microbiology and

storage life of VP pork, it was necessary to carry out a further experiment to determine if the surface chilling of fresh pork with a CO_2 chill tunnel is necessary when shipping large amounts of commercial, boxed product.

Temperature function integration offers a means of predicting the microbiological consequences of the temperature regime that a product experiences during various production processes without doing bacterial sampling (McMeekin et al., 1988). This technique involves the collection of product temperature histories with temperature data loggers and integration of the temperature data with respect to models describing the effect of temperature on the growth of various bacteria (Gill, 1986).

The objective of this study was to use the temperature function integration technique to determine if the CO_2 chill tunnel is a necessary part of the packaging process characterized in Chapter 2 when commercial, boxed pork loins are shipped to Japan in refrigerated trailers.

5.2. MATERIALS AND METHODS

5.2.1. Collection of product temperature histories

Product temperature histories were collected using MIRINZ-Delphi temperature data loggers (Tru-Test, Auckland, New Zealand). The data loggers were set to record temperatures between -20 and 40°C with an accuracy of ± 0.25°C and a resolution of 0.25°C at intervals of 2 minutes.

Three hundred fresh pork loins were packaged with the Cryovac packaging system characterized in Chapter 2. After passing through the CO_2 chill tunnel at 0.08 m/sec for 3 minutes loins were boxed with six pieces per box, and placed on pallets in the shipping cooler. During the 1 hour period in which the pallet (10 rows of boxes, five boxes per row) was being assembled, six temperature data loggers were placed in boxes of loins at different locations throughout the pallet (see Figure 5.1).

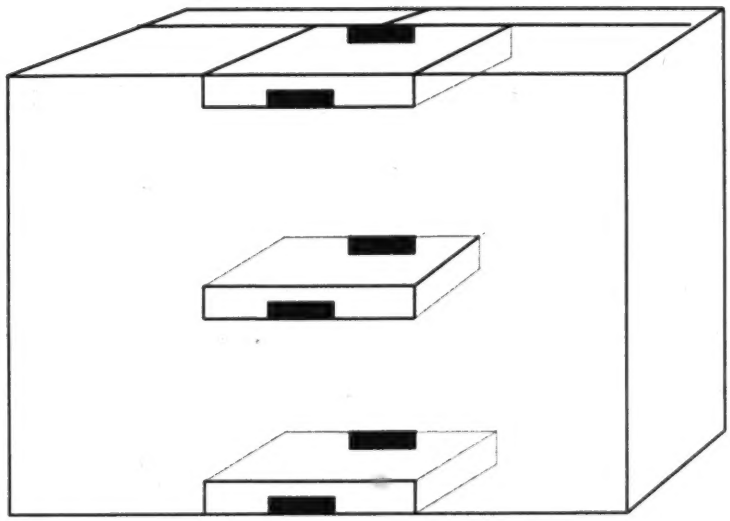


Figure 5.1. Diagram of a pallet with the six temperature data logger locations shown as black rectangles.

When 50 boxes of loins were placed on a pallet an additional 300 pork loins were packaged without being passed through the CO_2 chill tunnel. After exiting the heat shrink the loins were boxed and placed on pallets in the shipping cooler. Six temperature data loggers were placed in boxes of loins in identical positions to the six temperature data loggers for the 'chill tunnel pallet'. Both pallets were then loaded into a refrigerated truck trailer with a refrigeration unit set to deliver an air temperature of -1.1°C. The two pallets, each containing 50 boxes and 300 pork loins, were stored in the refrigerated trailer for approximately two days to simulate the first two days of transport to Japan. Following the storage period, both pallets were removed from the refrigerated truck trailer and relocated in the shipping cooler, the 12 temperature data loggers were removed, and the data was downloaded to a computer. Twelve product temperature histories and their corresponding data summaries were printed out.

5.2.2. Analysis of product temperature histories using temperature function integration

The product temperature histories were integrated with respect to models describing the growth rate of *Leuconostoc* spp. at different temperatures. The model has the form:

Eq. A. $y = (0.026\,x + 0.141)^2$ when x is between -2 and 30°C,

B. $y = 0.865$ when x is between 30 and 35°C,

C. and $y = 0$ when x is <-2 or >35°C,

where x is the temperature in °C and y is the growth rate expressed as generations h^{-1} (Gill et al., 1995). This model was chosen because LAB generally dominate the spoilage population in chilled, VP meats, and a species of *Leuconostoc* showed the most rapid growth rate of eight strains of LAB in a study done by Leisner et al. (1994). Assuming that LAB would become the dominant bacterial population in VP pork, it was deemed appropriate to carry out the temperature function integration analysis on the worst case assumption that the rapidly growing *Leuconostoc* spp. would emerge as the dominant

spoilage organism. The temperature function integration analysis was done assuming that the growth of *Leuconostoc* had commenced at the beginning of the simulated shipping process (Gill and Jones, 1992). The computer program used the *Leuconostoc* spp. model to estimate the growth of these organisms during the 2 day simulated transport period. The estimated growth of *Leuconostoc* spp. is expressed in generations and that number is included in the data summary printout. From the estimated growth of *Leuconostoc* spp., a storage efficiency factor was calculated for each of the six logger positions in each pallet. The storage efficiency factor is the percent ratio of *Leuconostoc* growth (in generations) during the 2 day simulated transport period as estimated by the computer program to the growth of *Leuconostoc* spp. (in generations) that would have occurred had the surface temperature of the boxed loins been at the optimum temperature of -1.5°C for the entire 2 day simulated transport period (Gill and Jones, 1992).

5.3. RESULTS

Table 5.1 shows the estimated *Leuconostoc* growth, average temperature during simulated 2-day transport, and the calculated storage efficiency for each of the six temperature data logger locations in each pallet. No data is presented for the 'top-inside' location because the temperature data logger at this location exhausted its battery power during the experiment. Special attention should be given to the 'middle inside' location because it represents the geometric centre of a pallet. It is this location that has the greatest insulation from the circulating air of the refrigerated trailer. The average temperature during the 2-day simulated transport at the 'middle inside' location for the pallet containing product that had been passed through the CO_2 chill tunnel (CT) was approximately 5.4°C cooler than the temperature at the identical location for the pallet containing product that was not passed through the CO_2 chill tunnel (NT). *Leuconostoc* spp. growth was 2.5 generations lower and storage efficiency was 40.3% higher for the

Table 5.1. Average temperatures, estimated *Leuconostoc* growth, and calculated storage efficiencies during 2 days of simulated transport in a refrigerated trailer.

	Chill Tunnel			Non Chill Tunnel		
LOCATION IN PALLET	Average Temperature (°C)	Growth of *Leuconostoc* (generations)	Storage Efficiency[1] (%)	Average Temperature (°C)	Growth of *Leuconostoc* (generations)	Storage Efficiency[1] (%)
TOP	-	-	-	3.9	2.6	17.5
INSIDE						
TOP	0	1.0	52.4	2.3	2.0	23.3
OUTSIDE						
MIDDLE	-0.1	0.9	53.7	5.3	3.4	13.4
INSIDE						
MIDDLE	-0.4	0.8	61.0	4.0	2.7	17.2
OUTSIDE						
BOTTOM	0.2	1.0	48.3	1.9	1.7	27.3
INSIDE						
BOTTOM	0.6	1.2	41.8	1.4	1.5	31.6
OUTSIDE						

- = exhaustion of temperature data logger battery power.

[1]Storage efficiency (% ratio of growth of *Leuconostoc* spp. calculated for a product temperature of -1.5°C throughout the period of simulated transport: growth calculated from the product temperature history).

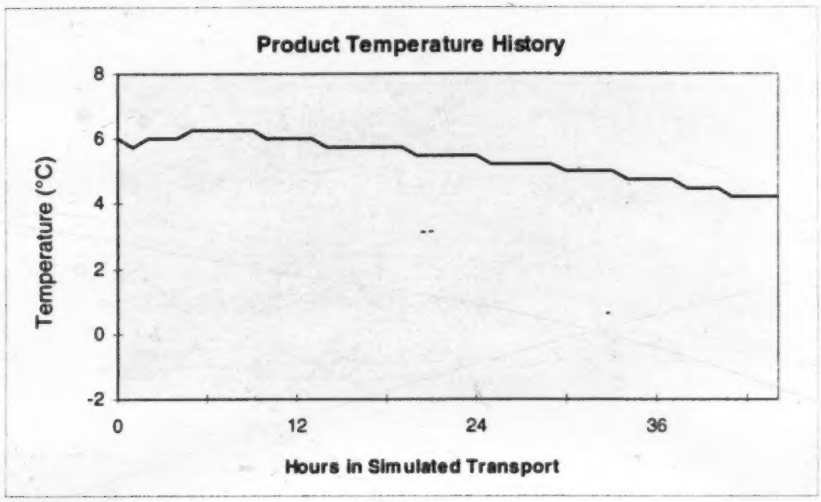
CT product compared with the NT product for the 'middle inside' location. Furthermore, the average temperature and estimated *Leuconostoc* spp. growth during the 2-day simulated transport were lower at all locations in the CT pallet; consequently, the storage efficiency at all locations in the CT pallet were lower than for the NT pallet. The 'outside' position on each of the three levels (top, middle, bottom) for both pallets showed lower average temperatures and higher storage efficiencies at all locations except for the bottom position of the CT pallet.

Figure 5.2 shows the product temperature histories from the 'middle inside' position (geometric centre) for each pallet. This data illustrate the dramatically higher temperature regime experienced by boxed, VP pork loins at the central position of the NT pallet. In the NT pallet, loin surface temperatures were about 6°C and slowly declined to about 4°C within about 48 hours. Contrarily, the CT pallet loin surface temperatures were below 0°C initially and did not change appreciably over the time period of the trial.

5.4. DISCUSSION

This study was designed to test the hypothesis that an insulation effect caused by storage of large quantities of VP pork loins in boxes on pallets for shipping after exiting the heat shrink, without exposure to the CO_2 chill tunnel, would result in a warmer temperature regime during transport to Japan compared with boxes of pork loins that had been passed through the CO_2 chill tunnel. Indeed, this hypothesis was confirmed as the temperature regimes experienced by boxed pork loins in the NT pallet were warmer than the CT pallet; with the most pronounced difference in temperature regimes occurring at the 'middle inside' location, where the insulation effect would be greatest. Ideally, to recreate commercial conditions, it would be necessary to pack a commercial consignment of boxed, CT and NT loins with temperature data loggers placed in boxes at strategic locations, into separate refrigerated truck trailers set to deliver an air

A)



B)

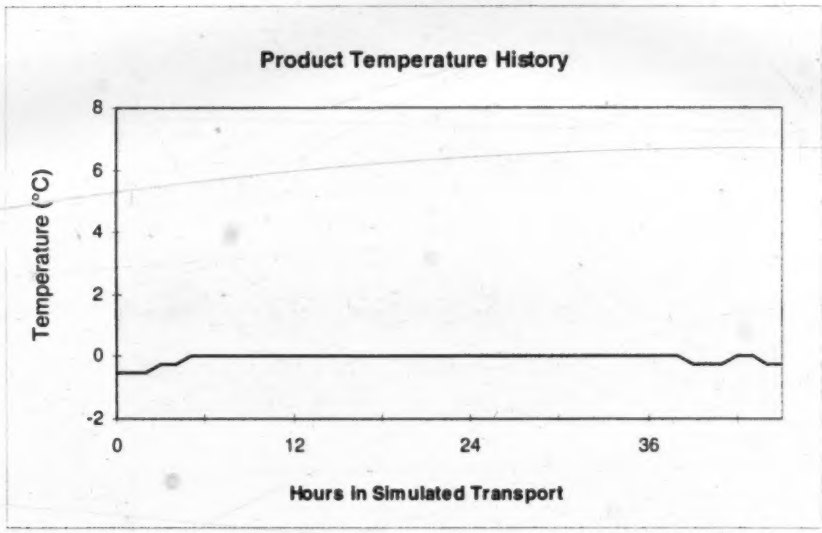


Figure 5.2. Product temperature histories showing the surface temperature of fresh, boxed pork loins during simulated 2-day transport in a refrigerated trailer. A) 'Middle-inside' location (geometric centre) of pallet that contained product that was not passed through the chill tunnel. B) 'Middle-inside' location (geometric centre) of pallet that contained product that was passed through the chill tunnel.

temperature of -1.1°C and held for approximately 14 days, to simulate actual transport to Japan. However, due to economic constraints, this was not feasible.

This experiment showed the dramatic difference in temperature regime experienced by pork loins that had not been passed through a chill and pork loins that were conveyed through a chill tunnel and packaged on single pallets for a 2-day simulated transport. It is reasonable to assume that if a refrigerated trailer was loaded with a commercial consignment of product not passed through the CO_2 chill tunnel and shipped to Japan, the insulation effect would be even more dramatic than that observed in this experiment. This would result in either a similar or warmer temperature regime during the 14-day transport period, having a deleterious effect on the microbial quality of the product.

Temperature function integration can be used to quantify bacterial growth when bacterial sampling is impossible or impractical to carry out. In this experiment temperature function integration was used to predict the negative effects of the much warmer temperature regime experienced by the NT pallet during simulated transport. The calculation of the storage efficiency for each temperature data logger position in the two pallets provided a means of reinforcing the beneficial effects of the CO_2 chill tunnel. This type of reinforcement is especially useful when dealing with industry management because it allows management to observe the effects of warmer temperature regimes on bacterial growth and storage life without microbial sampling. It appears that the CO_2 chill tunnel is vital in removing the heat imparted to the surface of fresh pork loins by the heat shrink and is therefore necessary to maintain a temperature regime as close as possible to the optimum during transport to Japan.

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6. General Conclusions

Export markets have become extremely lucrative for Alberta pork producers, generating over $940 million in 1995 (Canadian Meat Council, 1995). Much of the income generated through export markets for Alberta pork producers is derived from shipping pork to the United States and Japan. The extended storage life afforded by VP technology has allowed Alberta pork producers to reap the rewards of shipping fresh, unfrozen pork to distant overseas markets, most notably with greater consumer acceptance and higher economic returns. However, recently Japanese importers have expressed concern about the quality of fresh Alberta pork, particularly concerns with insufficient storage life and the accumulation of large amounts of purge in the vacuum package. Improvement of fresh pork quality and increased storage life were cited in 1991 as the key factors in elevating the competitiveness of Alberta pork in export markets (Mehr, 1991).

Little information exists in the scientific literature pertaining to the time/temperature parameters experienced by fresh pork packaged with the technologically advanced process utilized by Alberta pork processors. Furthermore, the temperature profile of Alberta pork during shipment to Japan is largely unknown. The initial study in this thesis was designed to answer these questions. Monitoring of temperatures of fresh VP pork loins at various points during the packaging process showed that they were packaged with a surface temperature between 4 and 5°C and an internal temperature between 2 and 3°C. Upon exiting the heat shrink the surface temperature of pork loins was as high as 20°C. However, passing the VP pork loins through the CO_2 chill tunnel removes heat imparted to the pork loin surface during the fabrication procedure and by the heat shrink. Consequently, fresh pork loins are boxed for shipment with surface temperature of ca. -1°C which is near the optimum of -1.5°C. During shipment of boxed pork loins to Japan it was found that the average surface

temperature was between -0.2 and -0.4°C and remained below 0°C for most of the 14-day journey. From the initial study it is also clear that the surface temperature of VP pork loins remains near or below 0°C from placement in boxes in Red Deer until arrival in Japan. This temperature regime definitely complies with Good Manufacturing Practice and should be the objective of all meat abattoirs in the fresh meat industry.

Bacterial metabolism and growth are temperature dependent. Consequently, the lower the storage temperature the greater the storage life of fresh VP meats. The second study in this thesis was designed to determine if the cooler temperature regime of pork loins and butts that are passed through the CO_2 chill tunnel resulted in product with superior bacteriology quality. In addition the storage life of VP primal cuts, bacteriology and retail case life of aerobically packaged slices, and flavour attributes of primal cuts were determined to assess the effects of the CO_2 chill tunnel on product quality. Because freezing of meat is commonly thought to increase purge (Greer and Murray, 1991) and that the CO_2 chill tunnel freezes the first few millimeters of the primal cut surface. The amount of purge accumulation in VP CT and NT loins and butts was determined.

This study showed that the CO_2 chill tunnel did not have beneficial or detrimental effects on any of the quality parameters studied. These results were somewhat surprising because of the dramatically higher surface temperature, observed at the time of boxing, of primal cuts that were not conveyed through the CO_2 chill tunnel after exiting the heat shrink. Temperature data loggers placed in the boxes of loins and butts shipped to the Lacombe Research Station for vacuum storage showed that after *ca.* 24 hours NT loins and butts had cooled to approximately the same surface temperature as CT loins and butts. It was hypothesized that the surface temperature of CT and NT primal cuts converged within 24 hours because there is very little insulation effect from of the low number of boxes stored in the vacuum storage cooler for each replication of the study (10 boxes). Consequently, the warmer initial surface temperature of NT primal

cuts did not persist long enough to have an effect on bacterial growth during vacuum storage or subsequent aerobic retail display, resulting in similar vacuum storage life, based on odour, and similar retail case life, based on odour, for both CT and NT pork.

Under commercial conditions hundreds of boxes of VP primal cuts are loaded into refrigerated trailers and shipped to Japan. It was hypothesized that a refrigerated trailer loaded with boxed primal cuts that have not been passed through the CO_2 chill tunnel after exiting the heat shrink will experience a drastically warmer temperature regime during shipment to Japan. This would have severe microbiological consequences compared with boxed CT primal cuts. This could be due to the significant insulation effect by the large number of boxes holding the heat from the warm meat surfaces around the primal cuts. This prevents the cold circulating air from easy assess to the boxed primal cuts, particularly the central or inside boxes.

To test this hypothesis a scaled down experiment using two pallets (50 boxes of VP pork loins/pallet) simulating commercial transport to Japan was done. Boxes on one pallet contained CT VP loins and the other pallet contained NT VP loins. Product temperature histories obtained by placing temperature data loggers at strategic locations in boxes of each pallet, including the geometric centre where the insulation effect would be the greatest, showed that the surface temperature regimes experienced by the NT loins were significantly higher than the CT loins. Temperature function integration of the temperature histories recorded at each temperature data logger location showed that for all logger locations in the NT pallet the estimated proliferation of *Leuconostoc* spp. (LAB) was much higher than for the CT pallet. From the estimated proliferation of *Leuconostoc* spp. (in generations) storage efficiencies were calculated for each temperature data logger location. Again, the NT pallet showed much lower storage efficiencies, of up to ca. 40% lower at the geometric centre logger location, for all data logger positions compared with identical positions in the CT pallet.

From the results found in the 'pallet' experiment the original hypothesis was confirmed. A second hypothesis that using a number of boxes of primal cuts that results in an insulation effect similar to that found during the pallet experiment there may have been a significant difference in bacterial growth during vacuum storage at -1 and 2°C; potentially affecting other quality parameters such as retail case life.

Results from the second study showed that the CO_2 chill tunnel had little effect on the accumulation of purge in VP pork loins and butts. Due to the fact that freezing of muscle tissue is generally considered to cause increased purge in VP meat the results were somewhat surprising. These results were attributed to variability in individual meat samples because primal cuts for the study were chosen from normal daily production. Because a commercial process was being evaluated muscle quality was not used as a selection criterion (e.g. pH). Consequently, an experiment was carried out (Chapter 4) to determine the effect of the CO_2 chill tunnel on purge accumulation using pork loins that were selected for the study on the basis of similar muscle quality and pH. However, when using pork loins of similar muscle quality the CO_2 chill tunnel had no effect on purge accumulation. Again, these results were surprising and the results have been explained by the fact that the rapid freezing (<5 min.) of the surface of primal cuts by the CO_2 chill tunnel does not cause enough cell damage and distortion to account for increased purge when the tissue thaws.

Research carried out and reported in this thesis have shown the CO_2 chill tunnel had no effect on the quality parameters studied when there was a minimal insulation effect during vacuum storage. At this point the CO_2 chill tunnel appeared not to be an important part of the technologically advanced packaging process utilized by Alberta pork abattoirs. However, due to the results found in the 'pallet' experiment it was concluded that the CO_2 chill tunnel is a necessary part of the packaging process when there is a substantial insulation effect due to large numbers of boxes being packed around each other, which occurs during commercial transport to Japan. Interesting

future research would be to conduct a storage study, as done in Chapter 3 of this thesis, using many more boxes in the vacuum storage cooler to simulate the insulation effect that would occur in a commercial situation. However, meat costs may be a limiting factor for this type of study. The studies reported in this thesis have demonstrated the importance of differentiating between 'science' and 'commercial reality'.

6.1. BIBLIOGRAPHY

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Appendix 1. Scorecard used for sensory evaluation of vacuum packaged pork loins and butts stored at -1 or 2°C for up to 8 weeks.

Pork Flavour Evaluation

Name:_____ Date: _____

Sample Order:

Ref _____ _____ _____ _____ _____

OVERALL FLAVOUR INTENSITY

Ref

none ——|——————————|—————————— very strong

LIVER

none ——|—————————————————————— very strong

SOUR/ACID

none ——|—————————————————————— very strong

DAIRY

none ——|—————————————————————— very strong

METALLIC

none ——|—————————————————————— very strong

BITTER

none ——|—————————————————————— very strong

OTHER -_____

none ——|—————————————————————— very strong

Appendix 2. Mean[1] log counts and pH of pork loins vacuum packaged with or without the CO_2 chill tunnel and stored at –1°C in vacuum for up to 8 weeks and then stored under simulated retail display conditions at 4°C for up to 9 days.

Time in Vacuum (weeks)	Retail storage (days)	Chill Tunnel[2]						No Chill Tunnel[3]					
		Total Plate Count	LAB[4]	Enterics[4]	CFC[4].	STAA[4]	pH	Total Plate Count	LAB[4]	Enterics[4]	CFC[4]	STAA[4]	PH
0	0	2.7	1.4	0.8	2.0	1.3	5.73	2.9	1.5	0.8	2.3	1.2	5.71
	2	4.7	2.7	2.5	3.4	2.5	5.76	4.6	2.7	2.5	3.9	2.6	5.72
	5	7.7	6.3	4.8	6.6	5.5	5.65	7.6	5.9	4.4	6.1	5.4	5.57
	7	9.3	8.0	6.1	8.1	6.7	5.83	9.2	7.5	5.5	8.0	6.8	5.58
	9	9.2	7.9	6.1	7.4	7.1	6.08	9.2	8.3	6.5	7.7	7.6	5.77
2	0	2.8	2.0	0.9	1.9	1.2	5.60	3.9	3.2	1.8	2.9	1.7	5.68
	2	3.7	3.9	2.3	2.3	1.6	5.53	4.9	4.8	3.1	2.8	3.1	5.54
	5	7.1	6.8	4.5	4.5	4.6	5.44	8.2	7.4	5.0	6.0	5.3	5.61
	7	8.4	7.6	5.9	7.0	6.1	5.54	8.9	8.4	6.2	7.3	6.6	5.64
	9	8.7	8.0	5.9	6.8	6.2	5.52	9.3	8.6	7.3	7.7	6.4	5.56
4	0	5.0	4.9	1.5	2.4	1.3	5.57	4.5	4.5	1.3	2.2	1.2	5.25
	2	6.9	6.9	3.0	3.2	3.1	5.64	6.9	6.7	3.2	3.1	3.5	5.45
	5	8.3	8.3	4.2	5.6	5.1	5.49	7.9	7.8	4.1	4.1	5.2	5.59
	7	8.8	8.6	6.3	7.5	4.8	5.55	8.7	8.4	6.2	7.4	5.5	5.65
	9	9.0	8.8	5.8	7.9	5.8	5.45	9.0	8.6	6.4	8.3	6.7	5.59
6	0	6.5	6.4	2.7	3.1	1.7	5.79	5.8	5.6	2.1	2.0	1.8	5.68
	2	7.4	7.4	2.8	2.8	3.4	5.65	6.9	6.9	3.0	2.7	2.6	5.72
	5	8.2	8.0	3.9	5.3	4.4	5.69	8.2	8.2	4.4	4.2	4.2	5.82
	7	8.8	8.5	5.8	7.2	4.8	5.61	8.6	8.6	5.3	6.9	4.3	5.71
	9	9.1	8.7	5.9	7.8	4.8	6.10	9.0	8.7	6.1	7.4	4.5	5.91
8	0	6.4	6.5	1.9	2.9	2.0	5.55	6.5	6.4	1.8	2.9	2.9	5.63
	2	7.4	7.3	3.5	3.0	2.5	5.65	7.5	7.5	3.2	3.0	2.7	5.7
	5	8.3	7.4	4.5	5.6	2.7	5.63	8.4	8.4	4.2	6.9	4.7	5.78
	7	8.5	8.4	6.7	8.0	3.7	5.67	8.7	8.6	7.0	8.0	4.0	5.69
	9	9.0	8.7	6.8	8.4	3.9	5.84	9.0	8.8	7.1	8.5	4.0	5.73

[1] Mean log counts are averages of four determinations (2 samples x 2 replicates).; mean pH are averages of 12 determinations (2 samples x 3 readings x 2 replicates).

[2,3] Loins that were packaged with or without the chill tunnel, respectively.

[3] LAB = lactic acid bacteria; Enterics = *Enterobacteriaceae*; CFC = *Pseudomonas* spp.; STAA = *Brochothrix thermosphacta*.

Appendix 3. Mean[1] log counts and pH of pork loins vacuum packaged with or without the CO_2 chill tunnel and stored at 2°C in vacuum for up to 8 weeks and then stored under simulated retail display conditions at 4°C for up to 9 days.

Time in Vacuum (weeks)	Retail storage (days)	Chill Tunnel[2]						No Chill Tunnel[3]					
		Total Plate Count	LAB[4]	Enterics[4]	CFC[4]	STAA[4]	pH	Total Plate Count	LAB[4]	Enterics[4]	CFC[4]	STAA[4]	pH
0	0	2.6	1.2	0.4	2.4	1.5	5.41	2.3	1.2	0.3	2.4	1.4	5.46
	2	3.4	1.4	1.3	3.1	2.0	5.38	2.8	1.4	1.4	2.7	1.9	5.46
	5	6.8	4.8	5.2	6.8	4.7	5.42	6.6	5.0	3.9	6.6	4.9	5.52
	7	8.3	5.9	6.5	8.3	5.8	5.36	8.2	5.9	6.2	8.2	5.6	5.51
	9	8.9	6.8	7.1	8.9	6.6	5.39	8.8	7.3	6.6	8.8	6.9	5.48
2	0	4.0	3.9	1.6	2.1	2.0	5.63	4.7	4.7	2.7	2.9	1.7	5.58
	2	6.2	6.1	3.3	3.0	3.4	5.47	6.6	6.6	4.0	3.9	2.6	5.51
	5	7.7	7.7	5.2	6.8	5.7	5.66	7.9	7.9	5.9	6.9	5.8	5.63
	7	8.7	8.3	6.9	8.2	6.2	5.51	8.5	8.2	7.8	8.4	5.1	5.69
	9	9.1	9.1	7.1	8.6	6.6	5.47	9.1	9.0	7.9	8.5	6.0	5.58
4	0	6.3	6.2	2.7	3.5	1.2	5.54	6.5	6.5	2.6	3.6	1.3	5.63
	2	7.3	7.3	4.3	3.8	1.9	5.52	7.2	7.2	4.2	3.8	2.2	5.50
	5	8.1	8.2	6.1	6.2	3.2	5.41	8.0	8.0	5.0	5.9	3.2	5.38
	7	8.5	8.2	6.1	7.5	3.5	5.45	7.4	7.5	6.0	6.8	3.2	5.45
	9	8.3	8.3	7.2	8.2	3.6	5.64	8.6	8.3	7.3	8.3	3.6	5.85
6	0	7.0	6.9	3.7	3.2	<1.2	5.67	7.0	6.9	3.8	3.6	1.6	5.58
	2	8.1	8.1	4.8	5.1	3.4	5.50	7.8	7.7	4.5	4.7	2.4	5.45
	5	8.5	8.9	5.8	6.4	4.1	5.49	8.2	8.0	5.8	5.5	3.2	5.59
	7	8.6	8.5	6.2	7.0	4.6	5.69	8.6	8.6	6.4	6.4	4.1	5.44
	9	8.9	8.8	6.9	7.6	4.9	6.01	8.8	8.8	7.0	7.2	4.4	5.71

[1] Mean log counts are averages of four determinations (2 samples x 2 replicates).; mean pH are averages of 12 determinations (2 samples x 3 readings x 2 replicates).

[2,3] Loins that were packaged with or without the chill tunnel, respectively.

[4] LAB = lactic acid bacteria; Enterics = *Enterobacteriaceae*; CFC = *Pseudomonas* spp.; STAA = *Brochothrix thermosphacta*.

Appendix 4. Mean[1] log counts and pH of pork butts vacuum packaged with or without the CO_2 chill tunnel and stored at –1°C in vacuum for up to 6 weeks and then stored under simulated retail display conditions at 4°C for up to 9 days.

Time in Vacuum (weeks)	Retail storage (days)	Chill Tunnel[2]						No Chill Tunnel[3]					
		Total Plate Count	LAB[4]	Enterics[4]	CFC[4]	STAA[4]	pH	Total Plate Count	LAB[4]	Enterics[4]	CFC[4]	STAA[4]	pH
0	0	2.6	1.6	1.0	2.5	1.5	5.94	3.2	1.4	1.3	3.0	1.7	6.04
	2	4.8	3.4	3.0	4.2	3.0	6.0	4.5	4.1	2.9	4.2	2.5	6.4
	5	8.0	6.6	4.9	6.8	5.6	5.81	8.1	7.0	4.8	7.2	5.3	6.24
	7	9.4	8.3	6.4	8.1	7.8	5.77	9.5	8.8	6.4	8.4	7.2	6.25
	9	9.6	8.7	6.7	8.5	8.0	5.97	9.5	8.5	6.5	8.6	7.8	6.52
2	0	4.4	4.0	2.2	3.5	2.5	5.97	3.9	3.8	1.9	2.8	2.0	5.89
	2	6.4	6.3	3.1	4.2	3.5	5.89	5.4	5.3	3.0	3.8	2.9	5.74
	5	8.6	8.2	6.9	6.8	6.0	5.93	8.4	7.7	4.6	7.4	6.2	5.70
	7	9.4	8.8	6.8	7.6	7.6	6.02	8.8	8.3	6.6	8.2	6.9	5.75
	9	9.5	8.9	7.7	8.1	7.8	6.96	9.5	8.8	6.8	8.9	7.8	5.90
4	0	6.8	6.6	3.3	3.3	3.7	5.67	5.7	5.8	2.8	2.5	2.5	5.68
	2	7.95	7.9	4.0	4.5	4.4	5.80	7.2	7.1	3.3	3.3	4.9	5.69
	5	9.0	8.5	5.5	7.4	7.1	6.05	9.0	8.4	6.2	7.2	7.1	5.79
	7	9.4	8.5	7.8	8.4	7.4	6.61	9.1	8.6	7.6	8.8	8.9	6.05
	9	9.3	8.9	7.6	8.9	7.1	6.17	9.7	9.0	7.5	9.4	7.4	6.19
6	0	7.3	7.2	3.9	3.8	4.0	5.97	7.3	7.3	3.9	3.2	3.8	5.95
	2	8.2	8.1	4.4	3.7	5.1	6.3	8.2	8.1	4.2	3.9	5.2	6.08
	5	9.0	8.5	5.6	6.4	6.3	6.46	9.2	8.5	5.6	7.4	6.6	6.21
	7	9.3	8.8	7.5	8.1	6.7	6.36	9.1	8.6	7.6	8.1	6.7	6.21
	9	9.5	9.1	7.9	9.4	7.0	6.34	9.6	8.9	7.8	9.2	7.0	6.45
8	0	7.6	7.4	4.7	4.0	4.2	6.06	7.3	7.4	4.6	3.5	3.8	5.91
	2	8.1	7.9	5.9	5.4	5.1	6.07	8.1	8.1	5.2	4.3	4.5	5.97
	5	9.4	8.8	5.9	7.8	6.2	5.84	8.9	8.4	5.8	7.6	4.9	5.84
	7	9.7	8.8	8.0	9.3	5.6	6.30	9.5	8.7	8.1	9.3	5.2	6.40
	9	9.6	9.0	8.5	9.4	5.6	6.91	9.6	9.0	8.5	9.3	5.3	6.91

[1] Mean log counts are averages of four determinations (2 samples x 2 replicates).; mean pH are averages of 12 determinations (2 samples x 3 readings x 2 replicates).

[2,3] Loins that were packaged with or without the chill tunnel, respectively.

[4] LAB = lactic acid bacteria; Enterics = *Enterobacteriaceae*; CFC = *Pseudomonas* spp.; STAA = *Brochothrix thermosphacta*.

Appendix 5. Mean[1] log counts and pH of pork butts vacuum packaged with or without the CO_2 chill tunnel and stored at 2°C in vacuum for up to 6 weeks and then stored under simulated retail display conditions at 4°C for up to 9 days.

Time of storage in Vacuum (weeks)	Retail storage (days)	Chill Tunnel[2]						No Chill Tunnel[3]					
		Total Plate Count	LAB[4]	Enterics[4]	CFC[4]	STAA[4]	pH	Total Plate Count	LAB[4]	Enterics[4]	CFC[4]	STAA[4]	pH
0	0	2.8	1.6	0.6	2.6	1.4	6.04	2.8	1.6	0.5	2.6	1.5	6.12
	2	4.6	4.1	2.4	4.7	3.2	6.28	4.4	3.9	2.5	4.3	3.2	6.29
	5	8.2	7.2	5.9	8.2	6.5	6.01	8.1	6.7	6.0	8.0	6.4	6.07
	7	9.3	7.6	7.4	9.1	7.4	6.44	9.3	7.5	7.5	9.2	7.5	6.58
	9	9.1	7.2	7.2	9.0	6.9	6.48	9.6	7.9	7.4	9.5	7.2	6.63
2	0	5.9	5.9	4.1	3.7	3.1	5.96	5.7	5.6	3.7	3.2	3.2	5.82
	2	8.1	8.1	5.5	6.0	5.2	6.11	8.2	8.2	5.6	6.0	5.2	6.03
	5	8.7	8.8	6.9	8.1	6.1	6.38	9.1	9.0	6.8	7.6	6.5	6.17
	7	9.1	8.8	7.3	9.0	6.5	6.50	9.0	8.7	7.5	8.8	6.2	6.14
	9	9.6	9.6	7.6	9.4	7.4	6.82	9.5	9.5	7.7	9.3	7.1	6.56
4	0	7.7	7.6	4.0	4.4	3.7	5.77	7.3	7.3	3.9	4.2	3.4	5.81
	2	8.1	8.0	5.7	5.4	3.7	5.73	8.2	8.3	6.5	6.0	4.2	5.96
	5	8.9	8.6	7.0	8.4	5.0	5.88	8.9	8.6	7.4	8.0	4.9	5.94
	7	9.1	8.7	7.0	8.7	5.4	6.19	9.1	8.9	7.2	8.8	5.4	6.17
	9	9.6	9.2	7.7	9.4	5.8	6.38	9.6	9.0	7.8	9.3	5.2	6.16
6	0	7.2	7.2	5.2	5.2	3.1	5.98	7.1	7.3	5.2	5.0	3.4	5.94
	2	8.2	8.0	6.3	6.5	3.8	5.66	8.3	8.2	5.9	6.1	4.2	5.90
	5	8.7	8.6	7.5	8.0	4.7	6.12	8.9	8.7	7.0	7.4	5.0	5.81
	7	9.0	8.9	7.6	8.5	5.6	6.50	9.2	9.1	7.4	8.0	5.7	6.18
	9	9.3	9.2	7.8	9.0	5.8	6.94	9.3	9.3	7.7	8.8	6.1	6.35

[1] Mean log counts are averages of four determinations (2 samples x 2 replicates).; mean pH are averages of 12 determinations (2 samples x 3 readings x 2 replicates).

[2,3] Loins that were packaged with or without the chill tunnel, respectively.

[4] LAB = lactic acid bacteria; Enterics = *Enterobacteriaceae*; CFC = *Pseudomonas* spp.; STAA = *Brochothrix thermosphacta*.

Appendix 6. Mean[1] flavour intensity values for vacuum packaged pork loins stored for up to 8 weeks at -1°C.

Flavour Attribute	Storage Time (weeks)	Treatment				
		Reference[2]	Chill Tunnel[3]	No Chill Tunnel[4]	Hidden Control[5]	SEM[6] ±
Overall	0	4.8	5.52	5.73	5.18	0.35
	2	4.8	5.66	5.22	5.26	0.38
	4	4.8	5.47	5.87	5.95	0.39
	6	4.8	6.42	5.92	5.92	0.34
	8	4.8	5.62	5.63	5.02	0.35
Liver	0	1.36	2.9	2.52	2.33	0.37
	2	1.69	2.44	2.57	2.76	0.30
	4	1.68	2.97	3.22	2.37	0.46
	6	1.28	3.11	2.93	2.22	0.39
	8	1.8	3.18	3.3	2.65	0.59
Sour	0	1.25	2.27	2.91	2.67	0.33
	2	0.92	3.63	2.46	2.03	0.33
	4	1.32	3.1	2.95	2.11	0.47
	6	0.98	2.64	2.16	2.81	0.37
	8	0.83	2.49	2.29	1.89	0.34
Dairy	0	0.82	1.78	1.65	1.56	0.19
	2	0.79	1.56	1.46	1.63	0.16
	4	0.84	1.66	1.57	1.76	0.33
	6	0.81	2.12	1.85	1.66	0.28
	8	0.45	1.44	1.46	1.21	0.31
Metallic	0	0.94	1.68	1.82	1.65	0.22
	2	1.07	1.87	1.74	1.88	0.23
	4	1.07	1.75	1.91	1.71	0.29
	6	0.99	2.18	1.96	1.73	0.22
	8	1.26	1.64	1.49	1.81	0.17
Bitter	0	0.89	1.64	1.80	1.65	0.29
	2	0.87	1.99	1.70	1.57	0.25
	4	0.88	1.89	2.07	2.11	0.24
	6	0.90	2.14	1.78	1.77	0.25
	8	0.72	2.18	2.22	1.33	0.50

[1]Means are the means of 40 scores (10 panelists, 2 replications, 2 samples).
[2]Fresh sample marked as a reference sample for the panel.
[3]Sample was packaged with the use of the chill tunnel.
[4]Sample was packaged without the use of the chill tunnel.
[5]Fresh reference sample presented to panelists as a coded sample.
[6]Standard error of the means.

Appendix 7. Mean[1] flavour intensity values for vacuum packaged pork butts stored for up to 8 weeks at -1°C.

Flavour Attribute	Storage Time (weeks)	Treatment				
		Reference[2]	Chill Tunnel[3]	No Chill Tunnel[4]	Hidden Control[5]	SEM[6] ±
Overall	0	4.8	5.09	5.04	5.30	0.38
	2	4.8	5.58	5.36	5.17	0.40
	4	4.8	6.15	5.45	5.44	0.48
	6	4.8	6.09	5.61	5.66	0.42
	8	4.8	6.06	6.63	4.94	0.55
Liver	0	2.51	3.59	3.36	3.73	0.43
	2	2.24	4.48	3.13	3.25	0.62
	4	2.61	4.29	3.74	4.18	0.73
	6	2.71	4.83	4.03	4.23	0.70
	8	3.23	4.38	5.41	3.74	0.62
Sour	0	1.05	1.95	1.45	2.87	0.26
	2	0.93	2.34	1.96	1.83	0.37
	4	0.99	1.73	1.62	1.63	0.19
	6	1.00	2.50	2.26	2.15	0.21
	8	0.87	1.97	2.19	2.32	0.34
Dairy	0	0.86	1.97	1.82	1.58	0.22
	2	1.02	2.28	1.40	1.66	0.24
	4	0.81	2.40	1.80	2.14	0.41
	6	1.12	2.15	2.30	2.12	0.31
	8	0.50	2.06	2.86	1.61	0.37
Metallic	0	1.12	1.54	1.63	1.79	0.20
	2	1.06	1.97	1.71	2.10	0.51
	4	1.44	1.63	1.58	1.76	0.39
	6	1.31	2.33	2.13	2.00	0.26
	8	1.53	2.06	1.62	1.43	0.18
Bitter	0	0.98	1.59	1.73	2.02	0.27
	2	0.86	1.64	1.53	1.63	0.30
	4	0.99	2.64	1.64	1.47	0.32
	6	1.05	1.90	2.20	1.96	0.33
	8	0.76	1.96	1.66	1.85	0.37

[1]Means are the means of 40 scores (10 panelists, 2 replications, 2 samples).
[2]Fresh sample marked as a reference sample for the panel.
[3]Sample was packaged with the use of the chill tunnel.
[4]Sample was packaged without the use of the chill tunnel.
[5]Fresh reference sample presented to panelists as a coded sample.
[6] Standard error of the means.

Appendix 8. Mean[1] flavour intensity values for vacuum packaged pork loins stored for up to 8 weeks at 2°C.

Flavour Attribute	Storage Time (weeks)	Treatment				
		Reference[2]	Chill Tunnel[3]	No Chill Tunnel[4]	Hidden Control[5]	SEM[6] ±
Overall	0	4.8	5.95	5.41	5.17	0.32
	2	4.8	5.77	6.92	5.97	0.40
	4	4.8	6.33	6.33	5.40	0.52
	6	4.8	6.08	6.42	5.12	0.44
Liver	0	0.87	1.17	1.03	0.88	0.35
	2	1.46	1.55	2.97	1.36	0.43
	4	1.39	2.17	1.25	1.29	0.56
	6	2.18	3.18	3.33	1.98	0.77
Sour	0	1.97	2.59	1.54	2.28	0.45
	2	1.88	3.56	3.28	3.13	0.62
	4	1.78	4.49	5.33	2.53	0.79
	6	1.3	0.81	0.84	1.84	0.17
Dairy	0	0.31	0.61	0.54	0.34	0.23
	2	0.88	0.84	1.51	0.98	0.41
	4	0.94	0.93	1.09	0.98	0.28
	6	0.64	0.62	0.72	0.90	0.17
Metallic	0	0.76	1.21	0.68	0.73	0.27
	2	1.28	1.11	1.11	1.07	0.21
	4	1.09	1.39	1.85	1.21	0.40
	6	0.72	0.77	0.79	0.82	0.12
Bitter	0	0.48	0.73	0.21	0.50	0.33
	2	0.98	1.33	1.67	1.48	0.39
	4	1.07	1.16	1.07	1.15	0.27
	6	0.70	0.76	0.84	0.82	0.10

[1]Means are the means of 40 scores (10 panelists, 2 replications, 2 samples).
[2]Fresh sample marked as a reference sample for the panel.
[3]Sample was packaged with the use of the chill tunnel.
[4]Sample was packaged without the use of the chill tunnel.
[5]Fresh reference sample presented to panelists as a coded sample.
[6] Standard error of the means.

Appendix 9. Mean[1] flavour intensity values for vacuum packaged pork butts stored for up to 8 weeks at 2°C.

Flavour Attribute	Storage Time (weeks)	Treatment				
		Reference[2]	Chill Tunnel[3]	No Chill Tunnel[4]	Hidden Control[5]	SEM[6] ±
Overall	0	4.8	5.87	6.32	6.29	0.31
	2	4.8	7.10	6.46	6.89	0.59
	4	4.8	11.44	9.73	5.19	1.00
	6	4.8	7.15	10.07	6.20	2.62
Liver	0	3.22	3.12	3.85	4.40	0.50
	2	2.67	4.30	4.82	4.60	0.73
	4	2.79	3.31	4.31	1.91	0.70
	6	3.37	4.43	3.63	3.30	0.27
Sour	0	0.60	1.07	1.58	0.90	0.28
	2	0.65	1.76	4.07	1.61	0.31
	4	1.17	1.83	2.49	2.31	0.61
	6	1.77	1.80	1.10	2.27	0.00
Dairy	0	0.88	1.04	1.04	1.38	0.24
	2	0.82	2.23	1.66	1.66	0.58
	4	0.99	1.70	3.20	1.56	0.91
	6	1.47	2.72	2.70	1.23	1.61
Metallic	0	1.07	1.18	1.68	1.43	0.35
	2	1.07	0.98	1.21	1.36	0.19
	4	1.09	1.48	1.19	1.26	0.23
	6	1.53	1.50	1.07	1.53	0.42
Bitter	0	0.62	1.10	0.94	1.41	0.31
	2	1.20	1.32	1.38	1.70	0.45
	4	1.30	1.42	2.46	1.43	0.59
	6	1.13	1.75	1.17	1.13	0.41

[1]Means are the means of 40 scores (10 panelists, 2 replications, 2 samples).
[2]Fresh sample marked as a reference sample for the panel.
[3]Sample was packaged with the use of the chill tunnel.
[4]Sample was packaged without the use of the chill tunnel.
[5]Fresh reference sample presented to panelists as a coded sample.
[6] Standard error of the means.